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Complexity and Uncertainty in Human and Ecological Risk Assessment

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COMPLEXITY AND UNCERTAINTY IN HUMAN AND ECOLOGICAL RISK ASSESSMENT

by

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ABSTRACT
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Multiple interacting stressors in the environment present increasingly complex risks to human health. Too often, however, the data required for traditional risk assessment are either lacking or unavailable at the necessary spatial or temporal scale. In addition, assessment practices and management policies need to move away from single factor approaches in order to accommodate the reality of complex chemical mixtures and environmental stressors. Recent literature suggests that a paradigm shift is under way. This points to a need for the development of new techniques both for rapid data collection and flexible risk assessment strategies that can adapt to make use of readily available data. This dissertation presents two types of methods for improving the risk assessment process given these evolving challenges: predictive analytics and integrated effect-directed toxicity screening.

The first technique addresses the characterization of environmental health using toxicological screening tools. Environmental influences on ecological and human health are often studied using indicators that represent important risk components such as chemical contamination, hazards, exposures, and biological stress. Unfortunately, studies are frequently constrained by the lack of calibrated indicators constructed from standardized metrics.

The second technique is a novel method for population-level risk assessment that uses self-organizing feature maps (SOM) to generate multivariate clusters of cause-of-death and birth outcome metrics, in combination with the use of and supervised learning risk-propagation

modelling to evaluate predictability of available indicators. I apply this method to identify exposure-outcome linkages at the county level for Wisconsin, USA and civil divisions in Dobrogea, Romania; thereby providing a dynamic visualization of public health risk relationships with behavioral risk factors (*e.g.* smoking, heavy drinking) and environmental factors (e.g. land cover, nitrates and faecal coliform in drinking water). These risk relationships do not demonstrate cause-effect, but provide guidance for targeted investigations and for risk-management prioritization.

To investigate a unique way of measuring environmental health, a sediment contact assay using zebrafish (*Danio rerio)* embryos was adapted from Hollert *et al.* (2003) as an indicator of teratogenic stress within river sediments. Sediment samples were collected from Lake Michigan tributary watersheds. Sediment contact assay responses were then compared to prevalence of congenital heart disease (CHD) and vital statistic birth indicators aggregated from civil divisions associated with these same watersheds. Significant risk relationships were detected between variation in early life-stage (ELS) endpoints of zebrafish embryos 72 hour post-fertilization and the birth prevalence of human congenital heart disease and infant mortality. Examination of principal components of ELS endpoints suggests that variance related to zebrafish embryonic heart and circulatory malformations is most closely associated with human CHD prevalence.

This study demonstrates a novel application of effect-based toxicity testing for ecological and human health risk assessments. These results support the hypothesis that bioassays normally used for ecological screening can be useful as indicators of environmental stress to humans so as to expand our understanding of environmental – human health linkages. Finally, next steps and new directions for these lines of thinking are discussed.

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CHAPTER 1: ENVIRONMENTAL EFFECTS ON HUMAN HEALTH: A REVIEW FOR CHEMICALS OF EMERGING CONCERN IN THE GREAT LAKES

A Report to the Health Professionals Task Force of the International Joint Commission

INTRODUCTION

A key problem in the Great Lakes (GL) is the continued presence, as well as emergence, of toxic substances that may occur at levels above those considered safe for humans and wildlife. In addition to well-known "legacy" toxicants, there also exist chemicals of emerging concern released to the GL and subsequently detected in water, sediments, fish, and wildlife. To protect human and ecosystem health against future threats, researchers and policy makers require a better understanding of such substances with respect to their potential risks. Great Lakes contamination from pollution is a well-established issue. Concerns regarding GL contaminants often warrant fish consumption advisories, apprehension over drinking water quality, and limitations for recreational use of waters. Chemicals of Emerging Concern (CECs) are of growing interest to entities such as the International Joint Commission (IJC), who recently tasked the Health Professional Task Force (HTPF) with investigating the current state of knowledge regarding CECs, the GL environment, and human health. The review that follows was commissioned by the HTPF to investigate the state of published scientific knowledge regarding potential risks to human health posed by emerging chemicals of concern (CECs) in the Great Lakes (Dellinger *et al.* 2011). This report is one component of a larger effort to advise the International Joint Commission on CECs.

Chemicals of Emerging Concern comprise a vast array of potential search topics, for the sake of focus the review was conducted using a literature search of seven categories of CECs. In this review, we (Dellinger *et al.* 2011) provided general suggestions for adapting risk assessment techniques to the growing landscape of pollution exposures in the GL. It was beyond the scope

of the original report to conduct a full quality assurance/control review of the 271 publications reviewed in preparation for this work. Therefore, this review relies on the established peer-review process of scholarly academic journals to assure quality of the research and resources cited. A recent comprehensive review of CECs in the Great Lakes was conducted by Klecka *et al*. (Klecka *et al.* 2010). By comparison, the goals of the Dellinger *et al.* (2011) report to the IJC were: (1) to concisely characterize the diversity and complexity of research under the topic "chemicals of emerging concern"; (2) to explore the concepts of risk assessment for CECs, particularly as related to human health risk assessment; and (3) to evaluate whether this review of the literature can provide suggestions to risk assessors and policy-makers as how to proceed in dealing with CECs and human health.

The term “Chemical of Emerging Concern” (CEC) can be used to describe “new and emerging chemicals” (Klecka *et al.* 2010) but generally refers to a growing awareness of environmental contamination from chemicals that are poorly understood or unregulated. New understanding of such chemicals can initiate new concern over previously unknown effects along with concern over the risk that these chemicals may pose to the health of humans and ecosystems. In addition to this definition, the term CEC is sometimes used to describe any pollutant that is not considered a “legacy” contaminant (often more tightly regulated or banned chemicals such as Hg, PCBs, Dioxins, etc.). Although I provide a list of CEC categories below, these categories do not encompass all possible “emergent” chemicals that could be present in and contaminate the environment. Therefore, this categorization of CECs denotes the complexity of the topic as well as the importance of an increased understanding of these chemicals.

There is no standard list of chemicals of emerging concern but Table 1 describes a list based on the report, “Chemicals of emerging concern in the Great Lakes Basin: an analysis of

environmental exposures" (Klecka *et al.* 2010). The work by Klecka et al. (2010) was conducted to help inform the IJC regarding the peer-reviewed scientific studies and reports since 1997 regarding chemicals of emerging concern that may pose threats to water quality in the Great Lakes watershed. After reviewing the Klecka et al. (2010) report, we were contacted by the HTPF to investigate the state of knowledge regarding human health and CECs. Klecka et al. (2010) provides a comprehensive review of CEC-related topics, upon which this first Chapter expands. For this reason, and in order to maintain continuity for the IJC, we adopted their classification of CECs in our work, with some modifications to fit the scope of this paper.

Our initial report (Dellinger *et al.* 2011), as well as the report put forth by Klecka et al (2010) focused on 15th IJC biennial report categories which did not include nanomaterials, pathogens or genetically altered organisms, nor the legacy chemicals (for example mercury or PCBs). Since the subsequent chapters do not necessarily speak solely to CEC-related issued I have included a section on non-CEC chemicals to this document.

METHODS

A search was conducted for each of the CEC (Table 1) categories using *ISI Web of Science*®, an online academic citation index provided by the Thomson Reuters Company. This index provides access to multiple databases, cross-disciplinary research, and in-depth exploration of specialized subfields within an academic or scientific discipline. Additionally, any cited paper within the cited index is linked to any other literature (book, academic journal, proceedings, etc.) cites these works. Also, journals and/or papers can be selected based upon their citation frequency in the literature, which can be interpreted as a surrogate measure for their impact in a field. In this way, current trends, patterns, and emerging fields of research can be assessed. Papers recommended by colleagues and Task Force members, and governmental reports and risk assessments were also included in the review. We also included some

references from cited publications that were not found in *ISI Web of Science*® but were located in *pubmed*®.

Topics were queried first through keywords searches relating to the chemical names (*e.g.* "Alkylphenol Ethoxylates", "Alkylphenolic Substances", "Nonyl-phenols", or CAS#: 25154-52-3). If keyword searching did not yield adequate results, citation indexing (searching articles related to those identified as relevant) was used to acquire related studies. Citation indexing was also used to follow up if a particular study showed great relevance (see below).

A subset of peer-reviewed scientific publications from each of the CEC categories from Table 1 was selected to represent the state of knowledge regarding the occurrence of CECs in the GL and the risks these chemicals pose to human health. Additional studies from outside the Great Lakes were also included to provide a balanced review on risk for exposure, general occurrence in the environment and effects on humans. Some categories, such as PBDEs and Akylphenolic Substances, yielded hundreds of publications while others such as synthetic musks, yielded far fewer hits. Although government agencies have produced comprehensive risk assessments on most CEC categories such as synthetic musks (EC 2005a, b, 2008a, b; ECB 2005) these reports were not included in the ISI database. The selection criteria were developed based on discussions with the members of the IJC Health Professionals Task Force (HPTF). The criteria for including a given paper were as follows in descending order of priority:

<u>Prioritization Selection Criteria:</u>

1. Relevance to the chemical group being searched
2. Relevance to Great Lakes region
3. Relevance to Risk Assessment
    a. Risk of exposure
    b. Presence in environment

c. Effects of chemical on humans and relevant model systems

4. Year of publication (preference to most recent research)

RESULTS

I: Brominated Flame Retardants

Description

Brominated flame retardants are a class of additive halogenated flame retardants used in numerous commercial products due to their fire retardant properties. Polybrominated diphenyl ethers (PBDEs), for example, are added to plastics and foam products to make them difficult to burn. The Agency for Toxic Substances and Disease Registry (ATSDR) has published a Toxicological Profile for Polybrominated Biphenyls and Polybrominated Diphenyl Ethers (PBBs and PBDEs) in which they are described as persistent, bioaccumulative and of concern to human health. The ATSDR states that, "because PBDEs are added rather than reacted to the product, they could leave the product under ideal conditions and enter the environment" (ATSDR 2004). They suggest that this rarely happens (ATSDR 2004). More recent literature reviewed below, however, has observed PBDEs as well as other flame-retardants and their metabolites in sediments, biota, and humans.

This category may include Polybrominated Biphenyls (PBBs) Polybrominated diphenyl ethers or other BFRs (HBCD (hexabromocyclododecane) and TBBPA (Tetrabromobisphenol A)). These chemicals also undergo metabolic transformation in top predators (whales, bears) potentially altering biotransformation and toxicity (McKinney *et al.* 2006). PBDE and HBCD metabolites have also been found to bioaccumulate in ringed seals and polar bears (Letcher *et al.* 2009).

Discussion of Hazards

The potential hazards suggested from our literature search include reproductive and developmental toxicity, immunity toxicity, neurotoxicity and endocrine disruption in humans and wildlife. This has been suggested by epidemiological, laboratory, and field studies (Roze *et al.* 2009; Saegusa *et al.* 2009; Talsness *et al.* 2009a; Zhang *et al.* 2010). The literature on BFR pays particular attention to endocrine disruption. Alterations to thyroid functions associated with PBDEs and HBCD, such as increased serum levels of thyroxine (T-4) and decreased serum levels of triiodothyronine (T3), have been documented in both human cohorts and exposed lab animals (Saegusa *et al.* 2009; Talsness *et al.* 2009a, b; Turyk *et al.* 2008; Wang *et al.* 2010; Yang *et al.* 2009). PBDE metabolites may produce higher endocrine disrupting activity than PBDE parent compounds. Hydroxylated-PBDEs, especially, induce serious endocrine disrupting interactions (Song *et al.* 2009; Yang *et al.* 2009).

According to the ATSDR (2004) PBDEs have not been definitively associated with health related outcomes, but are of concern due to their increasing presence and potential for human exposure. Several studies (published after 2004) described the following health concerns in humans associated with BFR exposure: developmental deficits in children (Roze et al. 2009) male genitourinary (GU) conditions (Small *et al.* 2009), and alterations of thyroid function (Dallaire *et al.* 2009; Turyk *et al.* 2008; Wang *et al.* 2010). Another study, using NHANES data, found two BFRs; PBB-153 and PBDE-153, which were significantly associated with both diabetes and metabolic syndrome. Dose-response relationships appeared to differ between these two chemicals (Lim *et al.* 2008). Both Turyk et al. (2008) and Dallaire et al. (2009) measured PCBs as well as BDE congeners when investigating these associations and, although they made efforts to control for demographics and medical history, it's difficult to rule out the possiblity that the alterations in thyroid function were due soley to PBDEs and PCBs as opposed to other

contaminants that may not have been measured. Similar criticisms could be raised regarding the other studies cited above and below.

Discussion of Evidence for Exposure

As previously reported by Klecka et al (2009), the publications reviewed here indicate that Tetrabromodiphenyl Ether (BDE-47), is the most environmentally prominent PBDE found in Great Lakes samples despite discontinuation of Pentabromodiphenyl mixture production in 2004. In contrast to BDE-47, Decabromodiphenyl Ether (BDE-209) is still used in manufacture of electronic devices. BDE-209 finds its way into top predators such as falcons and eagles (Johansson *et al.* 2009; Park *et al.* 2009a, b; Potter *et al.* 2009; Venier *et al.* 2010). PBDE levels in falcons were strongly correlated with nest proximity to human populations. Furthermore, BDE -209, while accumulating in these top predators, undergoes metabolic debromination to the banned, lower-brominated PBDEs (Johansson *et al.* 2009; Park *et al.* 2009a, b; Potter *et al.* 2009). The half-life of BDE 209 in higher organisms appears to be relatively short (Park *et al.* 2009a).

Both PBDEs and other BFRs (particularly the current-use BFR hexabromocyclododecane or HBCD) accumulate in Great Lakes food webs. For example, Batterman et al. (2007) reported secular increases in PBDE in GL fish. Additionally, PBDEs and emergent BFRs were reported in the plasma of GL bald eagles. A statistically significant relationship was found between total PBDE concentrations and total PCB and p,p'-DDE concentrations, suggesting that these compounds share a common source, which is most likely the eagle's food (Venier *et al.* 2010). The concentrations of 13 currently used brominated flame retardants (BFRs) were recently analyzed in 16 sediment cores collected from the North American Great Lakes(Yang *et al.* 2012). Among them, 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE), decabromodiphenyl ethane (DBDPE), hexabromocyclododecane (HBCD), 1,2-dibromo-4-(1,2-dibromoethyl) cyclohexane

(TBECH), and hexachlorocyclopentadienyl dibromocyclooctane (HCDBCO) were more frequently detected than others. Overall, these emerging BFRs have much lower concentrations than polybromodiphenyl ethers (PBDEs) and dechloranes. Inventories of the five BFRs were found to decrease linearly (log-scaled) with the increasing latitude of the sampling locations. Furthermore, BFRS, particularly DBDPE and BTBPE, were found to increase exponentially in recent years (Yang *et al.* 2012).

HBCD was recently shown to bioaccumulate in benthic invertebrates of Lake Ontario. It has also been shown to accumulate in Great Lakes herring gulls and lake trout (Gauthier *et al.* 2009; Ismail *et al.* 2009) as well as other non-Great Lakes food webs (Letcher *et al.* 2009; Losada *et al.* 2009). Sediment compartments of Lakes Huron and Erie serve as reservoirs for the accumulation and slow transfer of hexabromobiphenyl (hexaBB) to the food web constituents of these lakes (Lim and Lastoskie 2011).

Human exposure to BFRs is highly probable as contamination increases in the GL region. However, it is unclear to what extent human exposure routes stem from Great Lakes sources such as fish consumption. Although BFRs have been shown to accumulate in food webs and sediments of the GL, it is suggested that PBDE exposure in humans may result from multiple contributors (Anderson *et al.* 2008; de Wit 2002; Imm *et al.* 2009; Schecter *et al.* 2006; Ward *et al.* 2008). Of particular interest are a variety of inhalation exposure scenarios involving indoor materials such as carpets, car seats, and pillows. Upon comparing blood serum levels to bromine levels of household items measured by portable X-ray fluorescence (XRF) Imm et al. (2009) determined pillows and car seats to be the strongest predictors of lipid adjusted PBDE. Nevertheless, subjects with the highest levels of PBDEs (90^{th} percentile) from the study reported consuming more sport caught fish.

Given the increasing levels of BFRs in GL fish it seems probable that fish consumption contributes at least partially to human PBDE and other BFR exposure. As seen on Table *2*, Anderson et al. (2008) reported weak correlations between PBDE serum concentrations and various exposure routes. It is worth noting that serum PCBs (for which fish consumption is a well-established exposure route) were moderately associated with sport fish ingestion whereas PBDEs showed a much weaker, albeit significant, association. This suggests that although PBDEs and other BFRs accumulate in fish tissues (Batterman *et al.* 2007; Ismail *et al.* 2009; Luross *et al.* 2002; Zhu and Hites 2004) fish consumption is only one of several important exposure routes for PBDEs.

Evidence for additional factors contributing to PBDE exposure is provided by Schecter et al (2006), a market study measuring concentrations of thirteen PBDE congeners in 62 food samples. Additionally, they estimated levels of PBDE intake from food for the U.S. general population by age and sex. In this study, fish exhibited highest levels of PBDEs compared to other foods (Table 3), but meat contributed the most to PBDE dietary intake. For the first year of life, human milk was the major contributor of PBDEs. Schecter et al. (2006) concluded that diet most likely is not the sole or even major source of PBDE exposure. Furthermore, they point out that although these PBDE levels exceed those found in European and Canadian studies the dietary exposures evaluated in their study fail to explain the 10- to 20-fold higher levels in blood and milk from the U.S. general population.

Conclusions: BFRs

The information reviewed above suggests that BFRs accumulate in Great Lakes food webs, persist in the environment and disrupt endocrine function. Some degree of human exposure to these chemicals appears likely. Potential exposure routes could include inhalation of particles from household items or consumption of various food items including sport caught

fish from the Great Lakes. Although the consumption of GL fish may be an important exposure route for some individuals, the reviewed literature suggests it is not the 'major' source. There were no studies or risk assessments that explicitly suggest humans are at risk to PBDE-derived health effects from fish consumption. However, future risk assessments could show otherwise.

The importance of fish in a healthy diet cannot be refuted. I therefore identify the need for comprehensive risk assessments for BFRs and GL fish consumption to evaluate the degree of 'concern' over this particular pathway for BFRs. The results of Anderson et al. (2008) and Schecter et al. (2006) imply that legacy chemicals such as PCBs and Hg remain the dominant hazards of fish consumption, but not enough information exists to make a definitive conclusion.

The literature reviewed here suggests a high potential for exposure in humans, probably through multiple pathways. Despite the lack of definitive health effects associated with BFRs, a variety of publications cited here demonstrate common hazards, particularly endocrine disruption. Despite the possibility that much of the human exposure to BFRs is not GL related this chemical group merits careful consideration. Further clarification of exposure pathways to humans will help to elucidate the degree to which this group of chemicals is a GL priority.

II: Alkylphenolic substances

Description

Alkylphenolic substances (APs) were introduced in the 1940s and constitute a class of non-ionic surfactants used in detergents, paints, pesticides, personal care products, and plastics. Nonylphenol ethoxylates (NPEs) and their metabolites are a class of the broader group of compounds known as alkylphenol ethoxylates (APEs). *Nonylphenol (*NP) is the most common 'basic' chemical of the alkylphenol family. Typically, NP is further reacted to produce NPEs, and other condensation resins. Due to their high performance and cost-effectiveness, NP derivatives have emerged as the preferred ingredient in numerous industrial processes and products.

Environment Canada provides a Priority Substances List Assessment Report on Nonylphenol and its Ethoxylates (EC 2001). Alkylphenolics are detected in both biotic and abiotic strata of the Great Lakes (EC 2001; Klecka *et al.* 2010).

Discussion of Hazards

The literature describes APs as weak endocrine disruptors; certain metabolites such as p-tert-Octylphenol (OP) are known to bind to estrogen receptors (Bechi *et al.* 2006; Gregory *et al.* 2009). In a study cited by the EC (2001) risk assessment, NP was 1000–100 000 times less potent than estradiol in stimulating estrogenic activity (Lee and Lee 1996). Effects on sexual development from some of these compounds are documented in both laboratory and field studies using fish and mammals (below).

Zebrafish exposed to wastewater effluent contaminated with APEs showed reduced egg production up to 89.6%, 84.7% and 76.9% (Zoller 2006). Populations of fish exposed to higher levels of APs and their metabolites show higher prevalence of feminized traits (Balch and Metcalfe 2006; Kortner *et al.* 2009; Mayer *et al.* 2007; Vethaak *et al.* 2005). The prevalence of feminizing effects in male fish is largest in small regional surface waters that receive runoff or wastewater discharges (Vethaak et al. 2005, Mayer et al. 2007). However determining the magnitude of the contributions attributable to APs relative to human hormones in the effluent may require additional studies. For example, Jobling et al. (2009) explored the hypothesis that endocrine disruption in fish is multi-causal, resulting from exposure to mixtures of chemicals with both estrogenic and antiandrogenic properties. Using hierarchical generalized linear and generalized additive statistical modeling they demonstrated that feminizing effects in wild fish could be best modeled as a function of their predicted exposure (based on wastewater effluents) to both anti-androgens and estrogens or to antiandrogens alone (Jobling *et al.* 2009).

Although limited information exists regarding human effects and exposure, an Italian study estimated dietary intake of NP, OP, and octylphenol ethoxylate (OPE) in adults of about 12, 0.1, and 0.1 μ g day(-1). These estimated intakes of NP and OP were much lower than doses associated with toxic effects in laboratory animals (Ferrara *et al.* 2005). However, this low risk may not apply to developing fetuses. To investigate the effects of these compounds on human development Belechi et al. (2006) observed estrogen receptor expression in first trimester human placentas using an in vitro model of chorionic villous explants. Their findings suggest that the human trophoblast may be highly responsive to NPs, which raises concern over maternal exposure in early gestation (Bechi *et al.* 2006).

A follow-up study to Belechi et al. (2006) observed effects from extremely low doses of p-NP on the placental release of cytokines (Bechi *et al.* 2010). This suggests possible greater risk for maternal exposure to NPs during pregnancy despite the previously mentioned weak effects using lab animals. Furthermore, absorption and distribution of NP in maternal and neonatal rat uteri is documented to be extremely rapid, and can easily pass though the placenta during pregnancy (Hong *et al.* 2004)

Discussion of Evidence for Exposure

Some evidence suggests that Europe is much more contaminated by APE compared to North America and developing countries (Berge *et al.* 2012). Lee *et al.* found that the hydrophobic metabolites of APs such as the non-phenols (nonylphenol (NP)) are likely to accumulate in sludge of wastewater treatment plants whereas the water-soluble metabolites are likely to enter the environment when discharged into lakes (Lee *et al.* 2004). However, Mayer et al. (2007) demonstrated accumulation of NP metabolites in aquatic biota of Cootes Paradise in Hamilton, Ontario by a transfer of alkylphenolic substances from sediments to biota and their accumulation in the invertebrate tissue, particularly the highly hydrophobic 4-NP. These

investigators suggest that ingestion of contaminated sediment is the predominant exposure pathway for these organisms, and since invertebrates are an important food source for many wetland organisms, occurrence of these substances in the invertebrate tissue likely contributes to high prevalence (83%) of intersex condition observed in white perch collected from Cootes Paradise (Mayer *et al.* 2007). Hydrophobic NP metabolites were also detected in osprey eggs from Chesapeake Bay and fish from the Great Lakes area (Schmitz-Afonso *et al.* 2003).

A study from Taiwan observed concentrations of 4-NP and 4-OP in 59 human milk samples and suggested food consumption behaviors could contribute to NP and OP exposure (G-W Chen *et al.* 2010). OP concentration was significantly associated with the consumption of cooking oil (beta = 0.62, $P < 0.01$) and fish oil capsules (beta = 0.39, $P < 0.01$) after adjustment for age and body mass index (BMI). NP concentration was also significantly associated with the consumption of fish oil capsules (beta = 0.38, $P < 0.01$) and processed fish products (beta = 0.59, $P< 0.01$). The food pattern of cooking oil and processed meat products from factor analysis was strongly associated with OP concentration in human milk ($P < 0.05$).

Chen et al. (2010) observed that food consumption (particularly the use of cooking oil) was an important exposure route for Alkylphenols in Taiwan. Interestingly, the mean NP concentration in human milk from the Taiwanese participants (4.47 lg/kg) was much higher than those reported in studies from other nations (Ademollo *et al.* 2008; Cheng *et al.* 2006). The authors suggest that this may be due to the elevated levels of Alkylphenol concentrations in Taiwanese rivers and sediments compared to sediment from countries such as Italy, Japan and Germany (Cheng *et al.* 2006). Additionally, the Italian study (Ademollo *et al.* 2008) demonstrated a positive correlation between NP in breast milk and fish consumption (assessed within the cohort via questionnaire). NPs have also been observed in cow's milk as well as human breast milk in Chinese and Italian studies (Dorea 2009; Lin *et al.* 2009).

Conclusions: APs

The EC (2001) report on NP and NPEs concluded that these substances are "toxic" as defined in Section 64 of CEPA 1999 and that options to reduce exposure should be investigated. The EC (2001) also cautions that other APEs such as OP and OPEs (also covered above) have many of the same physical/chemical properties, which make them likely candidates as replacement or alternative products for NP and NPEs. However, they also possess similar toxicological properties and greater estrogenic properties. Exposure to humans is particularly concerning when one considers the presence of NPs in breast milk and the vulnerability of developing infants. Not enough information exists to comment specifically on the potential for exposure to humans living in the Great Lakes Regions.

Given the spatial distribution of AP contamination (*e.g.,* near wastewater treatment effluents), one may expect a risk of exposure to humans, particularly from GL related sources. Furthermore, the above studies suggested bioaccumulation of AP metabolites and identified AP metabolites in human breast milk. Although weakly estrogenic, the potential for exposure to developing infants who may be susceptible to APs (and perhaps APs mixed with other estrogen-mimickers) warrants precaution. It is possible that APs present a significant health risk to human populations in the Great Lakes and other regions. Further information on additive or synergistic effects of endocrine disruptors and environmental AP occurrence is needed.

III: Perfluorinated Compounds

Description

Perfluorinated Compounds (PFCs) are used as surfactants in a variety of industrial processes and are ubiquitous in the environment. This is due to their widespread use over the past 50 years in a broad range of applications, including use as surface treatments for carpets, fabric, and leathers for stain resistance, as well as coatings on various paper products (Klecka et

al. 2010). Health Canada (HC) provides a risk assessment for perfluorooctane sulfate (PFOS), its salts and its precursors, which are forms of PFCs. Since PFOS is likely the ultimate perfluorinated degradation or metabolic product of the group of substances considered in their report, HC suggested that levels of this compound in human tissue provide a useful indicator of exposure to this group of substances from all potential sources (HC 2006). The complex structure of perfluorinated surfactants makes it difficult to predict their environmental behavior from an understanding of their physical-chemical properties (Klecka et al. 2010).

Discussion of Hazards

Endpoints considered in the Canadian PFOS human health risk assessment included histopathology effects in the liver, carcinogenicity as well as hematological and hormonal parameters in humans (HC 2006). Potential human health outcomes due to PFC exposures suggested in recent literature vary from reproductive and endocrine disruptive effects to immunotoxicity of lab animals. Many publications simply cite prevalence in human blood and environment as cause for investigation, rather than invoking concern over any specific effect. Nevertheless, PFCs seem to affect reproductive and endocrine function in laboratory animals and human cohorts.

Some studies have documented alteration of thyroid status in fish. For example, PFC exposure was found to alter gene expression in the hypothalamic-pituitary-thyroid axis of dosed zebrafish (Shi *et al.* 2008; Shi *et al.* 2009). Additional studies using fish revealed reproductive deficits including alterations to ovarian development as well as offspring deformation and mortality (Ankley *et al.* 2005; Du *et al.* 2009).

Other Studies from the literature search suggesting hazards to human health mostly involved endocrine function, reproduction, and development. PFCs were shown to easily pass the placental barrier in mother-neonate pairs raising concern over fetal development (Midasch

et al. 2007). A hospital-based cross-sectional epidemiologic study of singleton deliveries in Baltimore, Maryland observed small negative associations between low levels of both PFOS and PFOA concentrations and birth weight, ponderal index (mass/height index), and head circumference (Apelberg *et al.* 2007). A similar study from Japan indicated a negative correlation between *in utero* exposure to low levels of PFOS and birth weight (Washino *et al.* 2009). In a mother-infant pair birth cohort from Taiwan, the odds ratio of preterm birth, low birth weight, and small for gestational age increased with PFOS exposure [per unit: adjusted odds ratio (OR) (95%CI) = 2.45 (1.47, 4.08), 2.61(0.85, 8.03) and 2.27 (1.25, 4.15)] (Chen *et al.* 2012). Finally, PFCs were associated with fewer normal sperm in a cohort of Danish men (Joensen *et al.* 2009). Efforts to identify developmental deficits and effect thresholds have yielded suggestive – but limited – results (Bjork *et al.* 2008; Butenhoff *et al.* 2009a; Butenhoff *et al.* 2009b; Chengelis *et al.* 2009a; Chengelis *et al.* 2009b; Das *et al.* 2008)

Discussion of Evidence for Exposure

PFCs are known to accumulate in Great Lakes biota including lake trout, blue gill and birds. The predominant perfluorinated compounds detected in fish were PFOS (De Silva *et al.* 2011; Delinsky *et al.* 2009; Furdui *et al.* 2007; Sinclair *et al.* 2006; Ye *et al.* 2008). Furdui et al. (2007) recently reported the spatial distribution of various PFC contaminants in lake trout from the Great Lakes. Fish from Lake Superior contained the lowest total concentrations, while the highest levels were found in samples from Lake Erie. Fish from Lakes Ontario and Huron showed similar total concentrations, respectively, while samples from Lake Michigan were lower (Furdui *et al.* 2007).

Furdui et al. (2007) also reported significant correlations between PFOS concentration and trout body weight. This suggests that PFCs bioaccumulate and biomagnify in food chains like other persistent bioaccumulative toxics such as Hg and PCBs. This is further confirmed by

(Ye *et al.* 2008) who found median PFOS levels significantly elevated in piscivorous fish (88.0 ng/g) when compared with non-piscivorous fish (15.9 ng/g) in whole fish homogenates from the Ohio, Missouri, and Upper Mississippi Rivers.

Fish contamination of PFCs occurs inland within the Great Lakes region as well. A study by Sinclair et al. (2006) revealed elevated levels of PFCs (PFOS were the most prevalent PFC) in Minnesota samples of blue gill, with median concentrations of 47.0-102 ng/g at locations along the Mississippi River, 2.08 ng/g in the St. Croix River, and 275 ng/g in Lake Calhoun. These results suggest that PFC contamination in freshwater fish is not limited to areas with known historical PFC inputs.

Food, breast milk and water ingestion are thought to be important PFC exposure routes for humans (Berger *et al.* 2009; Trudel *et al.* 2008; von Ehrenstein *et al.* 2009). In a comprehensive assessment of consumer exposure to PFOS and PFOA Trudel et al. (2008) investigated PFC contributions from gender- and age-specific exposure scenarios. The study found that North American and European consumers are likely to experience ubiquitous and long-term uptake doses of PFOS and PFOA in the range of 3 to 220 ng/kg body weight per day (ng/kg(bw)/day) and 1 to 130 ng/kg(bw)/day, respectively. The greatest portion of the chronic exposure to PFOS and PFOA is likely to result from the intake of contaminated foods and drinking water (Trudel *et al.* 2008.)

The above findings support the notion that GL fish consumption could play an important role in human exposure to PFCs (especially PFOS). Studies from other regions have suggested this exposure route. A study from Sweden analyzed PFC levels in fish muscle tissue from edible species caught in the second largest freshwater lake in Sweden, Lake Vattern, and in the brackish water Baltic Sea (Berger *et al.* 2009). The authors calculated human exposure to PFOS via fish intake for three study groups, based on consumption data from the literature. The

results showed that PFOS intake strongly depended on individual fish consumption as well as the fish catchment area. Comparing these findings to the results from Trudel et al. (2008) suggests that Great Lakes fish consumption is a PFC exposure route that deems serious consideration.

Conclusions PFCs

The review presents relatively strong evidence for human exposure to PFCs. This exposure is likely due to oral exposure routes including food and water consumption. These chemicals are accumulating in Great Lakes fish and therefore could contribute to human exposure via fish consumption. The evidence for human health hazards from PFCs is inadequate for definitive risk assessment or even identification of specific hazards.

IV: Current-use Pesticides

Description

Klecka et al. (2010) reported that pesticides are often observed in surface waters, sediments, groundwater, and sometimes atmosphere. Many studies in the searched literature focused on surface, ground or drinking water, particularly when investigating human health risk (Ochoa-Acuna *et al.* 2009; Waller *et al.* 2010b).

Although herbicides and insecticides have different modes of action, the category "pesticides" often combines these two categories and other chemicals. In this review "pesticides" were treated as one broad category. This was done to keep the volume of literature manageable for review. Unfortunately this categorization, initially reported by Klecka et al. (2010) oversimplifies the diverse list of chemicals it encompasses. The pesticide category does help to conceptualize the issue of "agrichemicals" as a common risk category (theoretically unified through exposure routes more so than hazards). Many studies on this subject included multiple chemicals in their reports. Some reports group them as "agricultural contaminants" or

"agrichemicals" the environmental source of which is generally thought to be agricultural or urban runoff.

Discussion of Hazards

A disadvantage of grouping pesticides, or any diverse group of chemicals, is the increased challenge of identifying specific hazards associated with the group. A study from Indiana found that atrazine, and perhaps other co-occurring herbicides in drinking water, is associated with an increased prevalence of small-for-gestational-age infants (Ochoa-Acuna *et al.* 2009). Furthermore, a retrospective, case-control study using Washington State Birth Certificate and US Geological Survey databases found an association between maternal exposure to surface water atrazine and fetal gastroschisis (congenital fissure of the ventral abdominal wall), particularly in spring conceptions (Waller *et al.* 2010a). Studies like these support the notion that developing infants may be susceptible to pesticide-contaminated drinking water. Both of these retrospective cohort studies controlled for socio-economic and behavioral factors such as drinking and smoking. However, confounding stressors (air pollution, water quality, and other contaminants) were not well accounted for. This is a common problem with studies associating human health effects with CECs.

The above associations are, however, supported by laboratory studies showing cellular and hormonal alterations to exposed human tissues. Orton *et al.* (2009) tested 11 pesticides (isoproturon, diuron, linuron, 4-chloro-2-methylphenoxy acetic acid (MCPA), mecoprop, atrazine, simazine, PCP, trifluralin, chlorpropham, and bentazone) for endocrine disruption. Common effects from this study were antiestrogenic/antiandrogenic activity in a yeast screen, and inhibition of ovulation, accompanied by decreased testosterone production (Orton *et al.* 2009). Changes to in vitro human chorionic gonadotropin (hCG)-stimulated hormone production in ovarian follicles was also observed (Orton *et al.* 2009). Another study exposed

human MCF-7 cells to environmentally relevant concentrations of atrazine. The doses altered protein expression in the cells. Affected proteins included those regulating oxidative stress such as superoxide dismutase and structural proteins such as actin or tropomyosin (Lasserre *et al.* 2009).

It is also worth noting that many pesticides negatively affect amphibian and fish reproduction according to several papers and reviews (Jin *et al.* 2010; Langlois *et al.* 2010; Rohr and McCoy 2010). Many studies outline negative effects of atrazine and other herbicides on amphibian reproductive health such as sex ratios and gonadal function (Langlois *et al.* 2010; Rohr and McCoy 2010). Similar effects were found in laboratory-exposed rats (Abarikwu *et al.* 2010) and zebrafish (Jin *et al.* 2010). This suggests that amphibians could serve as important sentinels of human health especially when considering agricultural runoff and drinking water exposure.

Discussion of Evidence for Exposure

Atrazine and other agricultural contaminants are suspected to negatively affect human birth outcomes via drinking water exposure. The Indiana study cited above calculated monthly concentrations during 1996-2002 of nitrates, atrazine and other pesticides using United States Geological Survey's National Water Quality and compared them to monthly United States birth defect rates for live births from 1996 to 2002 using United States Centers for Disease Control and Prevention natality data sets. Elevated concentrations of agrichemicals in surface water from April-July coincided with higher risk of birth defects in live births with maternal last menstrual period occurring in the same period (April-July) (Winchester *et al.* 2009). These patterns, and those observed by Ochoa-Acuna et al. (2009) suggest a relationship between agricultural application/runoff and human exposure.

Agricultural runoff is suspected to contribute greatly to the presence of these chemicals in the aquatic environment as well as soils (Echols *et al.* 2008; Pham *et al.* 2000; Riederer *et al.* 2010). Additionally, many pesticides travel further in water than they do in air (Matthies *et al.* 2009). In a study of 10 remote inland lakes in Ontario, Canada the most frequently detected chemicals in lake water, precipitation, and air were α-endosulfan, atrazine, metolachlor, chlorpyrifos, chlorothalonil, and trifluralin, and α-endosulfan. Chlorpyrifos were the chemicals detected frequently in zooplankton (Kurt-Karakus *et al.* 2011).

Conclusions: Pesticides (Agrichemicals)

As previously mentioned, many studies investigate health concerns related to agricultural runoff but not necessarily specific agrichemicals. Nevertheless, evidence of cellular stress and endocrine disruption raise human health concerns. Since pesticides appear to occur primarily in surface and ground waters, the potential exists for human exposure via drinking water and food crops. Even water treatment can result in disinfectant by-products which may raise health concerns (Krasner 2009). Antibiotic resistance of pathogens is another cause for concern.

Many veterinary pharmaceuticals (sometimes referred to as agripharmaceuticals) occur in the environment presumably from agricultural runoff and the lack of treatment for animal waste. These chemicals could include pharmaceuticals (amprolium, carbadox, monensin, and tylosin) as well as natural and synthetic steroid hormones (Snow *et al.* 2010; Song *et al.* 2010). Chemicals such as pesticides and veterinary pharmaceuticals are manufactured with specific modes of action (*i.e.*, specifically designed to be biologically and physiologically active). In this way, and because they are associated with agricultural runoff, veterinary pharmaceuticals and pesticides should be considered an important group of agrichemicals that warrants serious consideration.

Human exposure data for these chemicals are well covered in the CDC Fourth National Report on Human Exposure to Environmental Chemicals (CDC 2009). From this report, it is clear that humans in the United States are exposed to several pesticides as evidenced by their presence in urine samples according to the National Health and Nutrition Examination Survey (NHANES). The risks from these chemicals could include but are not limited to: reproductive, genotoxic, carcinogenic, neurotoxic, and respiratory effects.

Discussions with the HPTF influenced this review to focus on point-source pollutants. The review conducted here does not allow for a representative perspective of the literature on the various chemicals that could be included in the pesticide category. In our report (Dellinger *et al.* 2011) we strongly suggested that further consideration be given as to how pesticides, herbicides, and agrichemicals are categorized and reviewed for future discussion.

V: Chlorinated Paraffins

Description

Chlorinated paraffins (CPs), technical mixtures of polychlorinated alkanes (PCAs), occur ubiquitously in the environment (Bayen *et al.* 2006). CPs are used as lubricants and coolants in metal cutting and metal forming operations and as secondary plasticizers and flame retardants in plastics. Polychorinated alkanes represent a difficult analytical problem because of the complexity inherent in industrial mixtures. The total number of possible congeners is unknown, but far exceeds 10,000 (Eljarrat and Barcelo 2006). CPs seem to behave similarly to persistent organic pollutants (POPs), leading several countries to impose regulations on the use of CPs. The U.S. Environmental Protection Agency identified Short-Chain Chlorinated Paraffins (SCCPs), for action plan development based on their presence in humans; persistent, bioaccumulative, and toxic (PBT) characteristics; use in consumer products; production volume; or other similar factors (EPA 2009).

Discussion of Hazards

According to the EPA action plan acute toxicity of SCCPs (C_{10-13}) is very low. There was no evidence of developmental effects in prenatal developmental toxicity studies in rats and rabbits (EC 1999; EPA 2009; UNEP. 2009).

The liver, kidney and thyroid are major target organs in repeated-dose studies with rats and mice. When administered by gavage, chlorinated paraffins (C_{12}, 60 percent chlorine) are carcinogenic in rats and mice of both sexes (NTP 1986, 2005). The underlying mechanisms for the carcinogenicity of SCCP in rats and mice are not clearly known.

Information regarding PCAs in the GL and their effects on humans is limited. PCAs show sub lethal developmental deficits in laboratory frogs as well as trout (Buryskova *et al.* 2006; Cooley *et al.* 2001). Trout exposed to the PCAs (whole fish concentrations 0.22-5.5 microg g(-1)) showed a diminished or no startle response, loss of equilibrium, and developed a dark coloration. Dark coloration, however, is an indicator of stress and may not be pathologically relevant. Histopathological lesions were also observed in the livers of trout from each exposure group. Cooley et al. (2001) suggested that PCA toxicity is inversely related to carbon chain length, as has been observed in similar studies investigating mammals. The concentrations in the fish from this experiment were at levels that have been reported in invertebrates and fish from contaminated sites in the Great Lakes.

Discussion of Evidence for Exposure

According to the EPA action plan the primary non-occupational routes of exposure to SCCPs include ingestion, both directly and through contaminated food, and dermal contact with products. Chlorinated paraffins have been isolated from human liver, kidneys, adipose tissue and breast milk. SCCP exposure could occur from sources far from their use and release due to

their potential for environmental transport (EPA 2009). For example, SCCPs have been found in breast milk samples taken from Inuit women (UNEP 2009).

SCCPs and Medium Chain Chlorinated Paraffins (MCCPs) were found to biomagnify from prey and predators in both Lake Michigan and Lake Ontario. Trophic magnification factors for the invertebrates-forage fish-lake trout food webs ranged from 0.41 to 2.4 for SCCPs and from 0.06 to 0.36 for MCCPs (Houde *et al.* 2008). Klecka et al. (2010) reported on several studies that detected CPs in sediment and water within the Great Lakes but the information is limited and our report to the HPTF failed to uncover new information on the occurrence of these chemicals in the environment.

Bayen et al. (2006) offered the following future research priorities for CPs: improvements in analytical methodologies (reducing the complexity of the analysis, producing reference materials and performing inter-laboratory studies); determining background levels of chlorinated paraffins in the environment and human populations (this question should be answered using quality assured analytical tools allowing the inter-comparison of data); and investigating the sources of CPs to the environment and to humans.

Conclusions

Information found relevant to risk assessment on CPs was relatively sparse. The above information suggests a moderate concern for human exposure in the GL. Even fewer studies were found regarding human health effects. Based on the literature search for this review, the database remains inconclusive regarding the potential for human health hazards of CPS. Further research, perhaps following the priorities set forth by Bayen et al. (2006) could allow for stronger statements regarding concern over GL CP contamination.

VI: Synthetic Musks

Description

Synthetic musks are used extensively in perfumes, cosmetics, detergents, cleaning products, and other personal care products. As such, they have found their way into the surface water, sediment, and biota of the Great Lakes basin particularly near wastewater effluents (Guo *et al.* 2009; Sumner *et al.* 2010). Unlike their natural counterparts, synthetic musks have physical-chemical properties similar to other hydrophobic and semi-volatile organics that are known to bioaccumulate and biomagnify in aquatic organisms (Klecka et al 2010).

Discussion of Hazards

The European Commission (EC) has recently compiled risk assessments for the following synthetic musks: musk xylene (CAS# 81-15-2) (EC 2005a), musk ketone (CAS# 81-14-1) (EC 2005b), 1-(5,6,7,8-tetrahydro-3,5,5,6,8,8-hexamethyl-2-naphthyl)ethan-1-one (AHTN) (CAS# 21145-77-7) (EC 2008a), and 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-2-benzopyran (HHCB) (CAS# 1222-05-5)(EC 2008b) in which predicted no effect concentrations (PNEC) were developed (Table 4*)*. These risk assessments generally extrapolate human health risk from rodent models.

Laboratory studies suggest that synthetic musks influence endocrine function. These compounds show weak estrogenic activity (Bitsch *et al.* 2002a; Schreurs *et al.* 2004; Schreurs *et al.* 2002). One study tested a variety of musk fragrances using the E-screen assay that demonstrated estrogenic activity. A statistically significant increase in proliferation rate of human MCF-7 breast cancer cells was detected for two nitro musks (musk xylene, musk ketone), a major metabolite of musk xylene (p-amino-musk xylene), and the polycyclic musk fragrance AHTN (Bitsch *et al.* 2002b). Other studies show developmental toxicity in mussels (Gooding *et al.* 2006; Luckenbach *et al.* 2004) and fish (Carlsson and Norrgren 2004b). The zebrafish model

has suggested both endocrine disruption and reproductive effects in response to synthetic musks (Carlsson and Norrgren 2004a; Schreurs *et al.* 2004; Schreurs *et al.* 2002). One study suggested that certain polycyclic musks, including AHTN and HHCB, induce the expression levels of hepatic estrogen receptor alpha and vitellogenin mRNA/protein and modulate expression levels of CYP3A4 mRNA in the livers of male medaka (Yamauchi *et al.* 2008). The actual health consequences to humans from synthetic musk exposure remain unclear based on this review.

Discussion of Evidence for Exposure

Concentrations of polycyclic, but not nitro musks, were detected in freshwater fish from two urbanized areas in the lower Great Lakes: Hamilton Harbour in western Lake Ontario, and the Detroit River and nearby western Lake Erie (O'Toole and Metcalfe 2006). Although Klecka et al. (2009) reported that musks occured most frequently in biota (as compared to water or sediment) there exists little other information regarding their occurrence in humans or human exposure. One study observed low levels of musks in human breast milk (Kang *et al.* 2010). Their presence in Great Lakes biota, however, suggests a potential for exposure via fish consumption.

Two studies have evaluated serum levels of synthetic musks related to cosmetic use. No dominant source of nitro musk uptake was observed, although their relationship to body surface area indicates cosmetic products applied to the skin as the likely origin of plasma concentrations (Hutter *et al.* 2010; Hutter *et al.* 2009). Based on residue patterns and accumulation features, another study concluded that the exposure characteristics for synthetic musks are different from those of POPs, and that the major source of exposure to synthetic musks is probably via dermal absorption or inhalation (Reiner *et al.* 2007). However, as their use increases and they enter the environment (for example, near wastewater treatment facilities) the potential for exposure via drinking water or food web accumulation could increase.

Conclusions: Synthetic Musks

As with CPs the literature on synthetic musks provided sparse evidence for either exposure or health effects within the GL. Based on the current information the risk to human health and potential for exposure in the GL seems low relative to the use of personal care products. It should be noted, however, that additive effects of mild estrogen mimickers such as synthetic musks and APs could alter such recommendations.

VII: Organic Wastewater Constituents/Pharmaceuticals

Description

For reasons of efficiency and operational synthesis, we consolidated pharmaceuticals and Organic Wastewater Constituents (OWCs) into one group for the HPTF review. These are two very diverse groups of chemicals and they often overlap one another in the literature. These categories are so diverse that keyword searches proved impractical. The most successful method employed for searching OCWs and Pharmaceuticals was to meta-search the papers that cited papers such as Kolpin et al. (2002). This method of searching yielded papers that studied both OWCs and pharmaceuticals, often together. The reason for overlap is that pharmaceuticals generally enter the environment through wastewater from human excretion or simply flushing pills down the toilet.

OWCs can include anything from human hormone metabolites, to APEs (already covered as a separate chemical group) to a variety of pharmaceuticals. Overall, most papers focused on the following drugs and OCWs: coprostanol (fecal steroid), sterols (plant and animal steroids), N,N-diethyltoluamide (insect repellant), caffeine (stimulant), triclosan (antimicrobial disinfectant), tri(2-chloroethyl)phosphate (flame retardant), and 4-nonylphenol (nonionic detergent metabolite already covered in the APs section).

Discussion of OWCs and Pharmaceuticals

Seven of the 29 OWC studies we reviewed focused on the effects and occurrence of Triclosan in the environment. Triclosan has broad-spectrum anti-microbial activity against most gram-negative and gram-positive bacteria. It is widely used in personal care products, household items, medical devices, and clinical settings. Due to its extensive use, there is potential for humans in all age groups to receive lifetime exposures to triclosan, and, indeed, triclosan has been detected in human tissues and in the environment (Fang *et al.* 2010). Triclosan is suspected to disrupt endocrine function as shown in laboratory studies using -Wistar rats of both sexes (Kolpin *et al.* 2002; Stoker *et al.* 2010).

Most of the published studies suggest low appreciable risk to human health from current environmental exposures of pharmaceuticals. However, Kumar *et al.* (2010) identified the following shortcomings in determining risk to human health from environmental exposure to pharmaceuticals: (1) Use of measured versus predicted pharmaceutical concentration, (2) Identification of pharmaceuticals-of-concern and compounds needing special considerations, (3) Use of source water versus finished drinking water-related exposure scenarios, (4) Selection of representative exposure routes, (5) Valuation of uncertainty factors, and (6) Risk assessment for mixtures of chemicals (Kumar *et al.* 2010).

Mercury: Prominent Legacy Pollutant

Toxic concentrations of methylmercury (MeHg) produce particularly tragic consequences for human populations, striking hardest at a society's youngest members (JA Dellinger *et al.* 2012a; Mozaffarian and Rimm 2006; Nakano 2010). Mercury contamination of modern fisheries is especially insidious because it creates a conflict between two important health concerns: decreasing exposure to methylmercury and maximizing the nutritional benefit derived from seafood consumption, especially during pregnancy. The International Joint

Commission (IJC) has designated mercury (Hg) as one of the "dirty dozen" persistent organic pollutants (POP) which should be eliminated from the Great Lakes (IJC 2006). In its elemental form, Hg bears very little toxicity unless inhaled. Unfortunately, natural processes mobilize elemental Hg into organic forms allowing it to negatively affect the health and development of organisms. Concern over this matter has led to extensive studies on mercury and its toxic effects.

Due to bioaccumulation and the vulnerability of aquatic ecosystems to organic pollutants, the primary vector for mercury contamination in humans is fish consumption (ATSDR 1999). The IJC reports that in the Great Lakes region, the exposure to methylmercury (MeHg) is almost exclusively through fish consumption. As a result, many health studies involving Hg as an organic pollutant require the analysis of fish tissues. Current policy makers have set reference doses for MeHg in fish tissues at ranges from 0.1 to 0.5 ppm with 0.5 ppm as the approximate concentration for the majority of organizations such as U.S.FDA, and the World Health Organization (WHO) (IJC 2006).

Polychlorinated Biphenyls: Prominent Legacy Pollutant

Polychlorinated Biphenyls (PCBs) are a group of synthetic organic chemicals with demonstrated human health hazards (Aoki 2001; Faroon *et al.* 2001a, b). There are no known natural sources of PCBs in the environment. PCBs enter the environment as mixtures containing a variety of individual chlorinated biphenyl components, known as congeners, as well as impurities (Faroon *et al.* 2001b). The International Joint Commission (IJC) has designated PCBs as one of the "dirty dozen" persistent organic pollutants (POP) which should be eliminated from the Great Lakes (IJC 2006). The manufacture of PCBs stopped in the United States in August 1977 because there was evidence that PCBs build up in the environment and may cause harmful effects (Faroon *et al.* 2001a, b).

There are no reports of structural birth defects in humans caused by exposure to PCBs or of health effects of PCBs in older children (ATSDR 2000). However, PCB exposure has been linked with endocrine disruption, immune dysfunction, diabetes and cancer (Aoki 2001; ATSDR 2000; Dallaire *et al.* 2009; Faroon *et al.* 2001a, b; Hertz-Picciotto *et al.* 2008; Mozaffarian and Rimm 2006; Wigle *et al.* 2008)

Dioxins: Prominent Legacy Pollutant

CDDs are a family of 75 different compounds commonly referred to as polychlorinated dioxins. These compounds have varying harmful effects (ATSDR 1998). CDDs are primarily released to the environment during combustion of fossil fuels and wood, and during incineration processes. The International Joint Commission (IJC) has designated CDDs as one of the "dirty dozen" persistent organic pollutants (POP) which should be eliminated from the Great Lakes (IJC 2006).

CDDs are found in all environmental media, most people are exposed to very small background levels of CDDs when they breathe air, consume food or milk, or have skin contact with materials contaminated with CDDs (Consonni *et al.* 2012). For the general population, more than 90% of the daily intake of CDDs, CDFs, and other dioxin-like compounds comes from food, primarily meat, dairy products, and fish. CDDs may be present at much lower levels in fruits and vegetables (ATSDR 1998).

Exposure to CDDs can cause reproductive damage and birth defects in animals and humans (ATSDR 1998; Laporte 1978; Mocarelli *et al.* 2000; Van den Berg *et al.* 2006). The results of oral animal studies suggest that the most sensitive effects are immune, endocrine, and developmental effects. It is reasonable to assume that these will also be the most sensitive effects in humans (ATSDR 1998). CDD exposure has also been linked to teratogenic disruption of cardiovascular development using zebrafish models (Heideman *et al.* 2005; Jenny *et al.* 2012).

DISCUSSION AND CONCLUSIONS

Summary of Hazards: CEC Categories

Reviewing the literature of CECs from published epidemiologic investigations to governmental risk assessments reveals an overwhelming number of chemical variations within the CEC categories each with varying potential effects. This is despite the paucity of data regarding human exposure and health effects for many CECs. For these reasons, Tables 5 and 6 are meant to convey a generalized view of the CEC landscape without overstating the hazard and exposure potential of the larger list of chemicals (and health effects) inferred within. Table 5 demonstrates two facts regarding hazard identification of CECs: 1) all categories contain chemicals that have shown biologic and/or mechanistic effects that could lead to negative health outcomes, 2) many of the chemicals within these categories have been associated (not necessarily causally linked) with negative health outcomes. Depending on the amount and nature of exposure, these lists demonstrate many potential hazards to human and ecological health from CECs. This list could be expanded by adding the potential hazards from legacy and unknown pollutants.

It is important to consider that observations of perturbed biologic pathways (through toxicity testing) are separate from observing health outcomes. Biological systems may undergo perturbation from chemical agents and stressors without exhibiting outcomes such as disease. New toxicity testing techniques allow for quantification of the extent to which chemical contaminants may disrupt biological systems, which could lead to disease. Studies that investigate the probabilistic relationship between toxicity tests and the occurrence of disease in human populations could shed light on the complexities of exposure and hazard.

Biologic activity of CECs and other chemicals is often noted in risk assessments (ATSDR 2004; EC 2001, 2005a, b, 2008a, b; ECB 2005; EPA 2009). Yet, these mechanisms are not necessarily connected to any specific health outcome. One could argue that evidence of

biological activity (plus evidence of exposure) but no evidence connecting these chemicals to specific health outcomes is great cause for concern. The saying "absence of evidence is not evidence of absence" comes to mind. The fact that humans are exposed to many of these chemicals (Table 6), which are shown to disrupt biological pathways and yet no specific connections to health endpoints are reported raises the concern that risk assessments of these chemicals are lacking (as opposed to thoroughly concluding that public health is unaffected).

Summary of Exposures: CEC Categories

Aside from difficulties recording and assessing morbidity in human populations, identification of potential hazards for chemicals can be fairly straight-forward thanks to animal, *in vitro*, and *in vivo* studies. Exposure characterization and modeling, particularly for humans, presents challenges that increase uncertainty and obstruct identification of causal links to health endpoints. A point of contention among risk assessors is whether or not CECs or other pollutants occur at biologically significant levels in the environment. A more apt question to ask is; do the contaminant levels, combined with each other and the factors influencing exposure pathways, result in a biologically significant exposure to humans and ecosystems? As discussed below, I suggest current protocols fall short of addressing this question.

Table 6 demonstrates that some form of CEC exposure to humans occurs for all categories. In summary, the literature shows that human exposure to CECs is likely. As discussed above, most CECs have been shown to induce a variety of biological effects. Regrettably, characterization, identification, and modeling of the important exposure routes (either in the GL region or abroad) remain serious impediments to risk characterization for any chemical contamination. For example; total serum PBDEs are weakly (but significantly) correlated with sport fish ingestion (Anderson et al. 2008). PBDEs are known to accumulate in fish (Batterman *et al.* 2007; Gauthier *et al.* 2009; Ismail *et al.* 2009; Tomy *et al.* 2004). Yet,

sleeping pillows and primary vehicle seat cushions were the strongest predictors of log lipid-adjusted blood serum PBDE concentrations in a GL sport fishing cohort of 38 households (Imm *et al.* 2009). This identifies fish consumption as an important, but not major source, of PBDE exposure in humans. Similarly, Trudel et al. (2008) suggested contaminated food ingestion is the major, but not only, pathway leading to exposure to PFOS and PFOA in the general public. Future risk assessments must accommodate complex exposure models in order to realistically evaluate the relationships between health outcomes and pollution exposure.

Unfortunately, it seems researchers and risk assessors lack the tools (or perhaps just the frameworks for applying existing tools) to investigate the role these chemicals play in human health risk. Without these tools, any understanding of the extent to which chemical contamination affects human health will remain elusive.

Impediments in Assessing Health Hazards

Our review for the HPTF (Dellinger *et al.* 2011) identifies seven CEC categories with each category containing multiple metabolites congeners, species, or related chemicals. Grouping the CECs in this way allows for easier construction an organization of discussion regarding this topic, but also presents the potential to over simplify what is truly a complex field of research.

A recent report to the EPA identified emerging contaminants and persistent, bioaccumulative, and toxic (PBT) chemicals that were not being considered in current Great Lakes contaminant-measurement programs (Muir *et al.* 2009). These programs evaluated 22,043 chemicals of interest. Of these, Muir et al. (2009) identified 610 probable PBT substances that should be considered for further study and measurement in the Great Lakes region. Furthermore, the authors estimated that 101 of the 610 chemicals have been measured in the Great Lakes region (as parent compound or degradation products) and about 47 (legacy organochlorines, PBDEs and perfluorinated alkyl acids) are on routine monitoring lists. This

leaves hundreds of un-measured, poorly studied candidates that warrant "concern". It's important to consider that this list could be longer if more information existed on the other 21,000 chemicals originally considered. It may seem that the term Chemical of Emerging Concern implies "new chemicals"; however, this term appears to more adequately define chemicals that researchers do not yet understand well enough to confidently assess risk. For this reason, these chemicals are "concerning".

The goals of risk assessment are clear. Researchers and policy makers around the world use risk assessment to characterize the nature and magnitude of health risks to humans and ecological receptors from chemical contaminants and other stressors that may exist in the environment. This information is then used to assist in determining strategies for protecting humans and the environment from stressors or contaminants (EPA 2008). The following steps, which are modified from legislative language and the National Research Committee (NRC 1983), are generally accepted as the risk assessment process: Hazard Identification, Exposure Assessment, Dose-Response Assessment, and Risk Characterization. Typically, this process is carried out considering one chemical at a time using risk quotients and toxicological reports (ASTDR 2004; EC 1999, 2001, 2005a, b, 2008a, b, c; ECB 2005; EPA 2008, 2009; NTP 1986, 2005).

The above description generally outlines the process by which researchers and policy-makers guide causal research, aid in decision-making, as well as justify policy and management decisions. The chemical-specific nature in which this process is traditionally carried out limits the ability to address (1) the enormous and growing lists of chemicals, (2) uncertainty regarding the effects of chemical mixtures, (3) the impacts of mixed and confounding stressors and (4) residual unknown or unmeasured risk factors. Uncertainty is a well-accepted concept in risk assessment. However, as the list of CECs grows, so grows the uncertainty as to whether or not risk assessments accurately or operationally capture and manage the actual risks. This begs the

question, “Do all risk assessments need to be chemical specific?” Historically, and from a legislative standpoint, the answer has been “yes”. For example: The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) of 1996 states that all pesticides distributed or sold in the United States must be registered (licensed) by EPA. Such licensed chemicals must be proven not to elicit any unreasonable adverse effects on the environment and humans. The chemical-specific nature of risk assessment is also required by Section 408 The Federal Food, Drug, and Cosmetic Act (FFDCA), which authorizes the EPA to set tolerances, or maximal residue limits, for pesticide residues on foods. The Clean Air and Clean Water Acts establish similar provisions which are typically met by setting limits to specific contaminants.

Consideration of the risk assessment protocols put forth by the EPA in addition to adapted approaches employed by published research suggests basic steps for assessing risk to both ecosystems and humans (Figure 1). By definition, Ecological Risk Assessment (ERA) uses a systems-based approach to evaluate stressors and risks to ecosystem-based endpoints such as fish assemblages, animal populations, and habitat quality.

Many studies focused on ecological risk couch their work within the context of the Clean Water Act, which expressed its goals as restoring and preserving the physical, chemical and biological integrity of the Nation's waters. Earlier work by Karr *et al.* (Karr 1991, 1986) established biotic integrity as an ecological endpoint that would allow risk assessors and researchers to investigate stress-response relationships within an aquatic ecosystem. More recent studies (Alberti *et al.* 2007; Bedoya *et al.* 2011; Novotny *et al.* 2005; Shandas and Alberti 2008) have employed risk propagation and systems approaches to investigate the complex relationships between stress and response within aquatic ecosystems.

The concept of stress-response, as represented in Figure 1, may appear over-simplified but it accommodates the complexity of real-world relationships between the responses (such as

biotic integrity) and the potentially varied and interactive stressors (such as land use, pollution, and habitat alterations). I observe that current human health risk assessment (HHRA) protocols applied in the recent literature (Dellinger *et al.* 2011), though they serve an important role, do not adequately employ these concepts. It seems the exposure assessment step can serve as an empirical bottle-neck for investigating all the potential stress-response relationships. This is in addition to the severe impracticalities associated with identifying all the relevant human responses without violating human rights (addressed below regarding the collection of relevant human health data).

With the exception of perhaps some epidemiologic studies, human health risk is generally not investigated using a systems-based, stress-response approach with population health endpoints. I view this as a missed-opportunity to answer the 'tough' questions about human health and the environment. Considering available literature, risk-propagation approaches that observe human health response at a population level within a dynamic ecosystem are most likely to identify how stressors such as mixtures of pollution could interact with other factors like geography and behavior. These interactions could modify human exposures in ways difficult to anticipate without an ecosystem-based approach. This emphasizes a need for innovative techniques that can model complex exposures to human populations (Figure 2).

The chemical-specific nature of conventional risk assessment is meant to reduce uncertainty with regard to cause-effect interpretations as well as to accommodate federal regulations as exemplified above. Focusing on chemicals one-at-a time produces more interpretable risk calculations and more identifiable remedial actions. Unfortunately, this specificity may only increase uncertainty that risks from cumulative, additive, and synergistic effects are identified. Furthermore, it can be difficult to accept that observed effects from

observational cohort studies associating human health risk to chemical stressors are from the chemicals measured and not other uncharacterized variables. A number of the observational studies used for this review included such limitations.

As described above, evaluating the risks of potentially toxic substances employs an empirical, dose-response based approach through monitoring of selected chemicals in various media and biological receptors. This has resulted in an extensive knowledge-base regarding the spatial and temporal status of selected chemicals in aquatic ecosystems such as the Great Lakes. The current review indicates that many CECs are present in ecosystems in and around the Laurentian Great Lakes. However, the continued lack of definitive effects data on those chemicals represents an obstacle to assessing risk, thereby limiting the ability of Regions, States, and Tribes to make sound risk and remediation decisions.

Reducing the uncertainties regarding adverse effects associated with exposures to potentially toxic chemicals is a high-priority issue. Chemicals of emerging concern, in particular, require a more predictive approach due to the lack of existing effects data. Since many of these chemicals are poorly understood a focus on prospectively assessing hazards may prevent unanticipated human health tragedies. Researchers may begin to fulfill this need by increasing efforts to use effects-based information to predict and evaluate toxicity of chemicals. This effects-based information could serve as to screen for potential toxicological outbreaks. The adoption, and acceptance of such techniques, may require a paradigm shift in both regulatory and research approaches.

Uses and limitations of Effect-Directed Analysis

An increasingly popular method for assessing risk to stream ecosystems from multiple toxicants is Effect Directed Analyses (EDA). In contrast to evaluating individual chemicals, EDA provides the advantage of assessing complex mixtures as they exist in the environment. This

method employs laboratory bioassays indicating effects on cellular, organism or population level, which are intended to link measureable effects of complex environmental samples to distinct toxicants. Many ecological risk assessments from the European Union employ EDA to bridge the gap between chemical contamination and ecological status (Brack *et al.* 2007). EDA is a form of toxicity testing similar to toxicity screening; similar (sometimes the same) assays are used. Generally speaking, toxicity testing and EDA are methods used to quantify toxic response of biological pathways (either whole organism, or at cellular, molecular or genetic levels). Toxicity testing such as EDA uses assays that focus on biological responses suspected or known to be caused by toxic stressors (*e.g.*, teratogenicity, genotoxicity, endocrine disruption, *etc.*). EDA assays are relatively rapid and inexpensive compared to other toxicity tests such as those seeking to evaluate observable outcomes using mammalian models.

EDA was employed by Keiter et al. (2009) to investigate the potential role of toxic substances in fishery declines within the Danube River. They applied bioassays to sediment samples, and evaluated cytotoxicity, toxicity to bacteria, endocrine disruption, zebrafish embryo toxicity and mutagenicity. The results of the study indicated toxic substances as a likely candidate for fish decline in the river. Keiter et al. (2009) were able to associate the afore-mentioned toxic effects with suspended particulate matter as well as effluents from pulp mills and sewage treatment plants. The zebrafish test was particularly telling as embryos exposed to sediments from the upper Danube revealed significantly higher embryo toxicity. The above study uses EDA in a unique fashion: tests normally used for screening were treated as risk variables (ecotoxicological hazard potential) to test the hypothesis that Danube fisheries were at risk due to the presence of toxic chemicals. I propose that measures of ecotoxicological hazard potential could allow researchers to conduct predictive risk assessments without relying on resource-intensive chemical screening techniques.

EDA techniques such as the zebrafish sediment contact assay developed by Hollert et al. (2003) and employed by Keiter et al. (2009) provide a comprehensive method to investigate native sediments and particulate matter without employing extraction procedures. These sediment contact assays can be used to estimate bioavailability of particle-bound lipophilic substances and their toxicity to vertebrate development. Tools such as these would provide a quantitative measure of sediment or water contamination for use in toxicological investigations.

In isolation, EDA tests tell little of which specific chemical contaminants induce the measured effects (*e.g.* which chemicals in the Danube induced fish teratogenicity). However, in conjunction with local knowledge and further research, including targeted chemical analysis and risk propagation modeling, EDA can help prioritize remedial action and hypothesis testing. The integration of EDA tools could augment the effectiveness of monitoring, and reduce the dependence on chemical screening and chemical profiling. These tools would thereby delay expensive chemical identification until such time as causal hypothesis testing is indicated.

Priority Area: Collect relevant human health data for environmental studies

In addition to developing new risk assessment tools it is necessary to increase the availability of relevant human health data for the purposes of epidemiological study and HHRA. Such programs would regularly collect human-health data to facilitate study of human health/environment interactions and compile the information into (relatively) easily accessible databases. Some examples of current efforts on this front include the Wisconsin Pediatric Cardiac Registry (WPCR 2011) database, National Children's Study (NCS 2011), and Exposure Profiling (Malecki *et al.* 2006). Such databases would more easily allow for epidemiological studies on defined populations at risk.

The Commission for Environmental Cooperation (CEC; set up under the North American Agreement on Environmental Cooperation) has recently called for an increased effort in

measuring indicators of children's health. In a report entitled "Children's Health and the Environment in North America: a First Report on Available Indicators and Measures", the CEC describes cooperative work on children's health and the environment undertaken by the NAFTA-participating countries in three defined priority areas (CEC 2006). These priority areas include asthma and respiratory disease, lead and other chemicals, and waterborne diseases. The report presents thirteen indicators that fall within these priority areas. The indicators of interest included, but were not limited to: air quality parameters, asthma prevalence, pollutant release and transfer register data (such as the U.S. toxic release inventories), lead blood levels, pesticide residues on foods, and water quality parameters. The CEC (2006) reported that efforts by the three countries to compile these indicators revealed a number of data gaps and opportunities for improvement.

In 2002, the U.S. Congress allocated funding to the Centers for Disease Control and Prevention (CDC) to develop the National Environmental Public Health Tracking Program. The Council of State and Territorial Epidemiologists (CTSE) and CDC identified specific areas and indicators that should be evaluated to address the lack of understanding of environmentally related diseases. Future evaluations of these indicators should consider how well the indicator predicts human health and environmental conditions, and the adequacy of available data. In addition, the evaluators should also consider how best to standardize data collection and define the indicators (CDC 2011). Programs such as these are essential tools in the pursuit of more inclusive, accurate and integrated risk assessments.

Priority Area: Expand role the of screening assays in the risk assessment process

Ecological risk assessors face increasing demands to assess more chemicals, with greater speed and accuracy, and to do so using fewer resources and experimental animals (Ankley *et al.* 2010). Furthermore, new approaches in biological and computational sciences should be

applied to meet these challenges (Ankley *et al.* 2010). Ankley et al. (2010) propose the adverse outcome pathway (AOP) framework as a conceptual construct that describes the biological processes starting with direct molecular initiating event and an adverse outcome at a biological level of organization relevant to risk assessment (*i.e.* disease incidence). Understanding AOPs for pollutant-related hazards can increase the effectiveness of modern ecological risk assessment (ERA). I concur with these suggestions and propose that approaches such as AOP and EDA could serve to strengthen not just ERA but HHRA.

The National Research Council recently produced a report titled: "Toxicity Testing in the 21st Century: A Vision and a Strategy" (NRC 2007). The purpose of this report was to outline a vision for new approaches in toxicity testing using recent scientific advances. NRC (2007) cites substantial progress in the elucidation of cellular-response networks—interconnected pathways composed of complex biochemical interactions of genes, proteins, and small molecules that maintain normal cellular function, control communication between cells, and allow cells to adapt to changes in their environment. These advances could transform toxicity testing from a system based on whole-animal testing to one founded primarily on in vitro methods that evaluate changes in biological processes.

The opportunities presented by recent advances are important; however, the NRC (2007) points out that much refinement, development, funding, and years of research will be required before a new and efficient system of toxicity testing can be established. It is important to understand that shifting the paradigms in which assessors and researchers employ techniques to identify hazards and assess risk may also allow us to enhance these fields using relatively simple models such as zebrafish. Researchers can make these shifts now to form the first steps in establishing a much needed novel and efficient framework. To do this, the support of policy makers and funders will be required.

The development of techniques such as EDA and risk propagation for use in HHRA could help these protocols to account for complex mixtures of poorly monitored and poorly understood chemicals (i.e. real-life conditions). The next step in this process would be to calibrate promising *in-vitro*, *in-vivo*, and *in-situ* assays to respond to "real-life conditions". In this way, EDA and other screening techniques could have the potential to serve as a modeling tool to accommodate the needs identified in Figure 2 if properly applied. Also, risk-reduction goals, benchmarks, or standards for regulatory purposes relating to human health could be based on effect measures instead of specific chemical concentrations. A similar approach to conceptualize 'biotic integrity' (Karr 1991, 1986) used for ecological risk assessments and compliance with water quality standards for the Clean Water Act. In certain cases this could require the acceptance of non-apical measures as valid toxicity endpoints. Depending on the situation, it may not be necessary to run specific chemical analysis for all risk assessments. Thoroughly developed EDA responses combined with local knowledge (industry, geography, ecosystem and historical events) might be sufficient to make policy and management decisions and test hypotheses for human health responses

Well-established screening assays exist in the literature and are used for various applications. Some examples include fish teratogenicity (Hollert *et al.* 2003; Keiter *et al.* 2009; Keiter *et al.* 2010) and endocrine disrupting assays from the EPA Tier 1 screening list for Endocrine Disrupter Screening Program (EPA 2011). In our report to the HTPF, we recommended studies that investigate applicability of certain EDA assays to HHRA in conjunction with studies that investigate the ability of certain assays to respond to specific CECs in both laboratory and natural settings. The review of CECs, toxicity testing, and risk assessment suggests that these approaches will form an important framework for meeting the future challenges of both ERA and HHRA.

A review of the toxicity and CEC literature (Dellinger *et al.* 2011) indentifies four priorities: (1) to update toxicity testing for better integrated ecosystem and human health risk assessments, (2) to develop better risk assessment strategies for mixtures of CECs (which may occur at very low concentrations), (3) to develop new methods to better manage contaminants with poorly understood or unknown mechanisms leading to human health effects, and (4) move away from the legalistic cause: effect focus to better defining concern through a probabilistic approach that recognizes increased risk.

Conclusions and Recommendations: Chemicals of Emerging Concern

In addition to the literature cited above, the exposures to North Americans from many of the chemicals reviewed here are presented in the Fourth National Report on Human Exposure to Environmental Chemicals (CDC 2009). Additionally, governmental risk assessments from Canada, U.S. and the E.U. have investigated many of the reviewed chemicals at length. Although these works are useful, they did not provide a adequate framework for making the recommendations mandated by the IJC.

We (Dellinger *et al.* 2011) therefore found that the current exposure information and risk assessment methodologies do not as yet provide a basis for recommendations for the growing list of chemical contaminants in the Great Lakes basin. The CDC stated that the human health effects of most CECs at low environmental doses or at biomonitored levels from low environmental exposures are unknown (CDC 2009). This produces a dilemma when linking Chemicals of Emerging Concern to human health. Therefore, great discretion is required regarding how concern will be defined and interpreted in this context.

Current-use pesticides, which are primarily non-point source exposures, warrant a separate and more comprehensive consideration which has not included in this review. Also it must be noted that pharmaceuticals in wastewater (a CEC category under investigation by IJC,

HTPF, *et al*.) do not adequately comprehend non-point source pollution from the agriculture and other "run-off" sources. The findings from the contracted review and report agree with the SOLEC Biological Markers of Human Exposure to Persistent Chemicals Indicator #4177 which concludes that for the Great Lakes, human health assessment was/is "undetermined" and "not assessed".

The topic of pollution in the environment reemphasizes the 2006 recommendations to the governments on the Great Lakes Water Quality Agreement (GLWQA) from the IJC: "It is now time for a new Agreement with the requisite resources to produce significant results more rapidly so that the Great Lakes, as well as their tributaries, bays and connecting channels, are drinkable, swimmable and fishable for this generation and those to come" (IJC 2006). The extent to which CEC contamination threatens these goals (in addition to other important stressors such as: urbanization, climate change, invasive species, nutrient loading/eutrophication, microbial contamination, and sediment loading) remains unclear despite recent risk assessment efforts.

These realizations outline the need for action to adapt risk assessment, research, and policy to better inform the public regarding the concern for chemical contaminants in humans and the environment as well as potential progress towards a cleaner environment. Without these new directions in risk assessment, and the support of policy makers, risks to human health in the GL can neither be adequately determined nor managed. I concur with the sentiments of the 15th Biennial (IJC 2006) report and furthermore conclude that the methodologies, especially those regarding assessment of health effects, do not provide a complete framework for making recommendations on this topic and must be adapted to more closely match the methods evolving in ecosystem effects works. Chapters 2 and 3 present examples of how adaptive and

integrative strategies may enhance risk assessment to help address complex systems such as public health in the GL.

Table 1: List of Chemical of Emerging Concern (CEC) categories, based on the categories reiveiwed by Klecka et al. (2010).

Working List of CECs	
1: Brominated Flame-retardants	(BFRs, Polybrominated Diphenyl Ethers (PBDEs))
2: Alkylphenolic Substances	Alkylphenol Ethoxylates (APEs or APEOs) Alkylphenols (Aps) Nonylphenols (NPs)
3: Perfluorinated Compounds	Perfluorinated Sulfonates (PFSAs) Perfluoroctate Sulfonate (PFOS) Perfluorinated Carboxylates (PFCAs) Perfluorooctanoic Acid (PFOA)
4: Current-Use Pesticides	Herbicides, Insecticides, Fungicides
5: Chlorinated Paraffins	CPs, Polychlorinated Alkanes (PCAs)
6: Synthetic Musks	Fragrances, Personal Care Products
7: Organic Wastewater Constituents	Pharmaceuticals, Hormones, Disinfectant By-products, etc.

Table 2: Associations of PPBDEs, PPCBs, and DDE (ng g_1 lipid) with demographic characteristics and potential exposure routes for 472 participants. Drawn from Anderson et al. (2008).

Characteristic	*Spearman's correlation*	*Coefficient (r)*	
	∑PBDEs	∑PCBs	DDE
∑PCB ng g-^{1} lipid	0.17[a]	-	0.69[a]
DDE ng g-^{1} lipid	0.24[a]	0.69[a]	-
Sport fish ingestion years	0.15[a]	0.49[a]	0.35[a]
Sport fish meals/year	0.07	0.19[a]	0.29[a]

[a] $p < 0.05$.

Table 3: PBDE concentrations (pg/g wet weight) in market survey items from Schecter et al 2006. *NA = not available.

Medium	*N*	*Min*	*Mean*	*Median*	*Max*
Human Milk	62	31	1,916	968	21,359
Poultry	4	129	602	498	1,283
Beef	3	79	147	105	258
Pork	2	41	131	131	221
Milk	2	7.9	7.9	8.9	7.9
Cheese	6	9.8	185	97.6	683
Eggs	1	NA	85	85	NA
Margarine	1	NA	88	88	NA
Butter	1	NA	485	485	NA
Fish	24	11	1,119	616	3,726

Table 4: predicted no effect concentrations (PNEC) from the EC risk assessments (EC 2005a, b, 2008a, b).

Predicted No Effect Concentration Exposure Route	Musk xylene	Musk ketone	AHTN	HHCB
water (ug/L)	1.1	6.3	2.8	4.4
PNEC sediment (mg/kg dw)	0.3	0.5	1.72	2.0
PNEC 2^{o} consumer (mg/kg food)	1.0	0.3	1.1	3.3

Table 5: Perturbation of biological pathways and hazards suggested from a spring 2011 literature search of governmental risk assessments, epidemiology, laboratory, and wildlife studies and human health outcomes which have been associated with chemicals from each CEC category. Organic Wastewater Constituents were not included to avoid redundancy and over-generalization.

*Pesticides represent a broad range of chemicals with varied (and deliberate) modes-of-action. Recent discussions with the IJC have suggested that they be considered part of larger investigations of 'Agrichemicals' which could include agricultural pharmaceuticals.

Generalized CEC Categories	***Perturbed Biological Activity***	***Correlated Human Health Hazards***
1: Brominated Flame-retardants	Developmental, Immunological Neurological, Genetic, Reproductive, and Endocrine	Diabetes, Metabolic Syndrome, Thyroid Function, Child Development, Liver Damage
2: Alkylphenolic Substances	Endocrine, Reproductive, Carcinogenic	Breast Cancer Cell (MCF-7) Proliferation, Sperm Damage
3: Perfluorinated Compounds	Endocrine, Reproductive, and Developmental	Fetal Development, Birth Outcomes, Sperm Count, Cancer
4: Current-Use Pesticides	* Developmental, Immunological Neurological, Genetic, Carcinogenic, Reproductive, and Endocrine	*Fetal Development, Birth Outcomes, Cancer
5: Chlorinated Paraffins	Developmental, Neurological, Carcinogenic	None reported
6: Synthetic Musks	Developmental, Reproductive, and Endocrine	Breast Cancer Cell (MCF-7) Proliferation

Table 6: Evidence of exposure of CECs to humans based on a spring 2011 literature search of governmental risk assessments and published literature that observed chemicals from each category either in the environment or in humans.

*See above explanation of Pesticides.

Generalized CEC Categories	*Environmental Presence*	*Human Biomonitoring Presence*
1: Brominated Flame-retardants	Sediment, Biota, Food, Dust.	Blood and Breast Milk
2: Alkylphenolic Substances	Sediment, Water, Biota, Food.	Urine and Breast Milk
3: Perfluorinated Compounds	Sediment, Water, Biota, Food.	Blood and Breast Milk
4: Current-Use Pesticides	*Sediment, Water, Biota, Food.	*Urine and Blood
5: Chlorinated Paraffins	Sediment, Water, Biota, Food.	Liver, Kidney, Adipose, and Breast Milk
6: Synthetic Musks	Sediment, Water, Biota.	Blood and Breast Milk

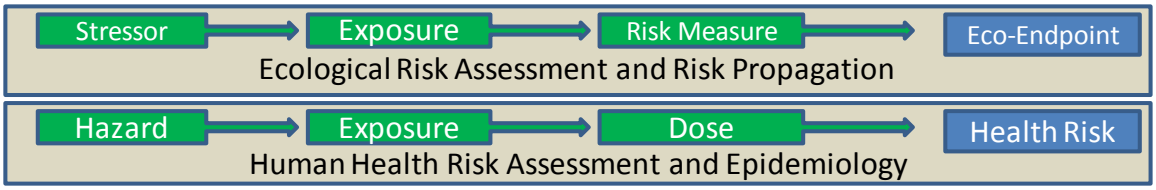


Figure 1: Conceptual model comparing human health risk assessment process to ecological risk assessment process both originally based on EPA risk assessment protocols.

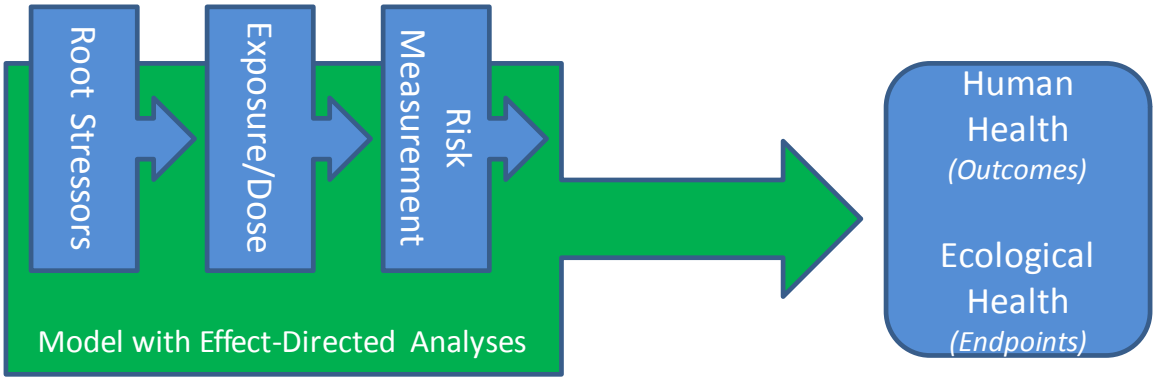


Figure 2: Concept illustrating the need for techniques that model exposure and mixed stressors within complex systems to allow for innovative, informative human and ecological health risk assessments.

CHAPTER 2: ZEBRAFISH SEDIMENT CONTACT ASSAY: A NOVEL TOOL FOR INTEGRATING HUMAN AND ECOLOGICAL RISK ASSESSMENTS

INTRODUCTION

Effect-Directed Analysis and the Case for Integrative Toxicity Screening

Evaluation of environmental health presents the challenging task of identifying useful quantifications (indicators) of environmental health (Dellinger *et al.* 2011; Sexton 2012; Sexton and Hattis 2006). Risk assessment must support the cost-effective investigation of environmental exposures with potential human health consequences (Strauss *et al.* 2010). Indicators of important risk components (e.g. chemical contamination, hazards, exposures, and biological stress) could be based on animal/biological models, epidemiological studies, or biomarkers, and would unify risk assessment across disciplines. Effect-directed analysis (EDA) bioassays constitute one approach that addresses all of these challenges (Dellinger *et al.* 2011).

The present study uses EDA as a screening tool for toxicity in human-inhabited communities and as a risk indicator relating stream teratogenicity to infant health. EDA is a category of methods developed as part of the European Union (EU) Watershed Framework Directive (WFD) for assessing risk to stream ecosystems from multiple toxicants (Brack *et al.* 2007). Instead of inferring specific causal relationships for toxic responses, EDA quantifies biological responses to environmental samples. This study will focus on the Zebrafish (*Danio rerio*) Sediment Contact Assay (ZSCA) developed by Hollert et al. (2003), which is one of the EDA techniques currently used for WFD assessments (Hollert *et al.* 2003; Keiter *et al.* 2009; Keiter *et al.* 2010). ZSCA is a comprehensive method for investigating native sediments and particulate matter without employing extraction procedures, and can estimate bioavailability of particle-bound lipophilic substances and their toxicity to vertebrate development (Hollert *et al.* 2003;

Keiter *et al.* 2009; Keiter *et al.* 2010; Kosmehl *et al.* 2006; Rocha *et al.* 2011; Strecker *et al.* 2011).

Congenital heart disease (CHD) is the most common birth malformation in infants occurring in 0.8-1% of infants (Hoffman 1995), and a leading cause of infant mortality (Botto and Correa 2003; Srivastava 2000). The etiologies of these malformations are largely unknown. Only about 2% of cases are due to known environmental causes such as viruses, drugs and toxins. The majority (about 73-85%) are attributed to multifactorial causes (Wigle *et al.* 2008), including gene-gene interactions, multiple gene or gene-environment interactions (Botto and Correa 2003). Studies also suggest a connection between environmental exposures and CHD, including a three-fold increase in hypoplastic left heart syndrome (HLHS) (a severe form of CHD) among the offspring of women exposed to solvents during pregnancy (Kuehl and Loffredo 2006; Loffredo 2000). Preliminary data from the state of Wisconsin have suggested an increased occurrence of HLHS within certain geographical areas of the state (Cronk 2004). In Milwaukee County, increased occurrence of CHD is associated with proximity to trichloroethylene emitters (Hanson-Morris and Pelech. 2006; Wigle *et al.* 2008; Yauck *et al.* 2003). Because of the heterogeneity of these defects, including differences in their embryogenesis, unique methods to investigate potential environmental risk relationships are needed (Botto and Correa 2003; Srivastava 2000; Wigle *et al.* 2008).

Public health indicators, collected as part of the registration of birth/death of infants by state vital records agencies, are usually analyzed in relation to socioeconomic status (SES) indicators (WDHS 2012b). All three indicators used in the present study (infant mortality, prematurity and low birth weight) have documented associations with lower SES. But these indicators may also directly or indirectly reflect environmental influences. For example, infant mortality and low birth weight are both higher among infants born with congenital anomalies

that may result from exposure to teratogenic agents (Botto and Correa 2003; JA Dellinger *et al.* 2008). However, it is also the case that exposures to certain teratogenic agents such as trichloroethylene may be more common in locations close to lower SES neighborhoods, and thus be one of the multiple factors leading to higher infant mortality or low birth weight.

In the present study, ZSCA is used to quantify sediment contamination and teratogenicity of watershed ecosystems. Findings represent the local risk of exposure (including risk to humans) to mixed chemicals and stressors. The following analysis provides evidence to evaluate ZSCA as a risk assessment tool, a measure of variations in environmental degradation, and its value as an environmental health indicator which could be associated with negative infant health outcomes such as CHD.

In this study, I hypothesize that fish teratogenicity will demonstrate a measurable response to sediment contamination, organic content, and pore water toxicity. Furthermore, I hypothesize that multivariate patterns of fish teratogenicity, averaged across city-bound watersheds, will describe increased risk of negative birth outcomes within the human population.

METHODS

Site Selection and Sampling

Sediment-contact assay using zebrafish embryos has been adapted from Hollert et al. (2003) to create a metric of teratogenic stress caused by watershed sediments. Sediments were sampled to represent watershed teratogenicity of Wisconsin civil divisions and Milwaukee ZIP codes (loosely referred to as cities). The ZSCA assay results were then compared to sediment contamination data and prevalence of human birth outcomes in the corresponding cities. Because ZSCA is a measure of teratogenicity, birth prevalence of congenital heart disease and

vital statistic indicators of infant health were selected as response variables potentially representing human teratogenic effects in the units sampled.

Sample sites were selected by examining city, township, ZIP code and minor civil division (MCD) boundaries over-laid upon the WIDNR level 5, 10-digit Hydrologic Unit Hierarchy (HUC - 10) boundaries using ArcGis 10 (©2001 ESRI) (Figure 3). Sediments were sampled from watersheds within the boundaries of 22 cities and four Milwaukee watersheds (the Milwaukee watersheds were joined to zip codes) to create a total of 26 human-populated sample areas, generally referred to as cities for brevity (Figure 3). Cities were prioritized by availability of health data and proximity to Lake Michigan. One upstream site and one downstream site (relative to municipal boundaries) within watersheds were selected to represent stream toxicity of the cities. If more than one watershed intersected the city boundaries, then extra sites were sampled to represent the contributing watersheds. We made no assumptions regarding the direction or magnitude of teratogenic differences between upstream and downstream sites. It was assumed, however, that an upstream sampling and downstream sampling were necessary to generalize the inputs and accumulation throughout the watershed as it related to city boundaries.

Each site consisted of three near-surface (0-5cm) sediment samples per transect using an Ekman dredge. Two transects (totaling 6 dredge "grabs") were sampled 10 meters apart to represent a site. Samples were stored in 100mL Nalgene® polypropylene jars and transported on ice. Samples were centrifuged at 680xg for 15 minutes to separate pore water from sediment and fine particulates. Pore water was transferred to a separate collection jar and stored at -20°C for toxicity testing using the RAPIDTOXKIT™ assay. Sediments were stored at -20°C in Ziploc® plastic freezer bags for toxicity testing and chemical analysis.

Health Data Collection

Data on occurrence of congenital heart defects were obtained from The Wisconsin Pediatric Cardiac Registry (WPCR). Operation of the WPCR was reviewed and approved by the lead institution (Children's Hospital of Wisconsin) and the other participating clinics and hospitals. Starting January 1, 2000 the WPCR registered families of infants with CHD (Hanson-Morris and Pelech. 2006) whose mothers were resident in Wisconsin at the infant's birth. Participating physicians from seven clinical centers throughout the state refer patients to the WPCR. Diagnostic exclusions include infants with an isolated patent foramen ovale (PFO) or patent ductus arteriosus (PDA), electrical conduction disturbances of the heart without an associated structural abnormality and acquired heart diseases, such as Kawasaki's, rheumatic fever or endocarditis. Diagnoses of registered patients are confirmed by clinical examination and either echocardiography, catheterization, surgery or autopsy. For purposes of this study, all CHD diagnoses were aggregated for analysis. Data collected through medical records or the WPCR questionnaire on minor civil division of birth residence were used to assign cases to the appropriate geographic unit. From 2000 through 2009, more than 5,000 CHD cases have been registered.

Data on infant mortality (death of a live born infant occurring in the first 12 months of life), prematurity (births of infants between 30 and 37 weeks of gestation), and low birth weight (infants born weighing less than 2500 g) were obtained through a data sharing agreement with the Wisconsin Bureau of Health Information (Table 7). Minor civil division recorded on the birth registration form was used in the analysis. As indicated above, data were aggregated by civil political boundaries due to the reliance on DHS birth data to scale birth outcome occurrence. Spatial analysis, used ArcGis 10 (©2001 ESRI), to map watersheds onto political boundaries.

Sediment Chemical Analysis

Chemical analysis was developed and performed using pesticide grade solvents. A total of 27 sediment samples from 11 different cities (including all the Milwaukee samples) were analyzed for the following chemical contaminants: polychlorinated biphenyls (PCBs), dioxins, and polybrominated diphenyl ethers (PBDEs). Sediment samples were lyophilized and sifted (1mm screen) then subjected to organochlorine extraction in hexane using an ASE 150 accelerated solvent extractor (Kiguchi *et al.* 2006). Dioxin/PCB and PBDE fractions were collected via multilayer column chromatography (Liu *et al.* 2006).

PBDE samples were analyzed via UHPLC MS/MS using a Dionex RSLC 3000 UHPLC and an AB Sciex 4000 QTrap (Mascolo *et al.* 2010). Limit of quantification was 0.42ng/g sediment for each of the measured PBDE congeners (28, 47, 99, 100, 153, 154 183, and 209). Total PBDEs were calculated from the aforementioned congeners. Dioxin/PCB samples were analyzed using a Thermo Ultra Trace GC with PTV inlet, AS3000 autosampler, and ECD detector. Initial oven temperature was 180^{o}C for 1 minute then 2.5^{o}C per minute to 270^{o}C for 30 minutes (total run time 65 minutes). PTV inlet temperature 300^{o}C, splitless time 0.8min, split flow 30ml/min. H_2 carrier flow rate 1.5ml/min. ECD base temperature was 270^{o}C with ECD at 300^{o}C. Makeup (N_2) flow rate was 50ml/min. Ten mcl samples were injected onto Dioxin DB column 60X0.25mm, 0.25micron film thickness. Standards curves were created with r^2 values >0.98 for individual coplanar PCB and dioxin standards. Injections of known standard mixtures of PCDFs and non-coplanar PCBs were performed with the method to ensure no co-elution with measured standards within the method. Limit of quantitation for each of the PCB congeners (77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, and 189) and dioxin congeners (1,2,3,4,7,8,9-HpCDD and OCDD) were 0.029ng/g sediment. Total PCBs and Dioxins were calculated via summation of the aforementioned congeners.

It was necessary to assess oxygen demand and organic content to evaluate the effects of toxicity from factors related to, but not necessarily causally linked to, chemical contaminants. To investigate the role of anoxic sediment conditions on embryo toxicity, total oxygen demand (TOD) was assessed. TOD was recorded over five days for a subset of samples (2g in 300 mL water) using an YSI 5000 oxygen probe (Delzer 2003). Organic carbon content was determined using loss of ignition method (Dean 1974). These data were transformed to adjust for heteroscedasticity (log10 for quantities and arc-sin for proportions) prior to analysis.

Zebrafish Sediment Contact Assay

Sediment samples were prepared 2 hours before testing. Twenty four-well microtiter plates were filled with 0.5g of native defrosted sediment and covered with Embryo 2, 'E2' water (Nusslein-volhard and Dahm 2002). Sediment and water were warmed to 27°C prior to embryo exposure.

Zebrafish embryos were collected from breeding populations (Ekkwill strain) at the Children's Environmental Sciences Health Core Center (CEHSCC). At 2 hours post fertilization (HPF), embryos were added to the prepared microtiter plates at a density of one embryo per well and exposed to the native sediment for 46 hours at 27°C. At 48HPF embryos were transferred to clean water and observed for 15 Early Life-stage (ELS) endpoints using a dissecting microscope. Embryos were returned to the incubator (27°C) for an additional 24 hours and observed again at 72HPF for ELS endpoints. The following ELS endpoints were recorded at 48HPF and 72HPF: General Characteristics, (Dead, Un-hatched, Underdeveloped, and Hypopigmentation) Edemas, (Cardiac, Yolk, Abdominal, and Cranial) and Structural Malformations (Body Axis, Somite Development, Tail, Eyes, Heart, Circulation, and Head). All sediment samples were tested at least 3 times ($n \geq 18$) per experiment (13 experiments were

run in total). Each plate contained a negative control row (Aerated, buffered water without sediment) of embryos (n=6, per plate).

Pore Water Toxicity Assay

RAPIDTOXKIT™ tests were used to evaluate the response of *Thamnocephalus platyurus* larvae to pore waters by measuring feeding inhibition relative to controls (MicroBioTests 2008). Feeding inhibition was tested twice per sediment pore water sample (N =164). Mean feeding inhibition was calculated for analysis as a measure of pore water toxicity associated with the sediments (N = 82).

Treatment of Data and Analysis

Statistical analyses were conducted using JMP 9.0 (©2010 SAS institute). Sediment characteristics data were examined for divergence from normal distributions and were transformed using logarithmic or arcsine transformations as necessary to adhere to the distributional assumptions of statistical tests. For both RAPIDTOXKIT™ and ZSCA, mean responses of the control organisms for that day (per replicate) were subtracted from test values to create a response relative to untreated conditions to normalize data for comparison across different dates. Inhibition of *T.platyurus* was calculated by dividing the difference in control and treatment responses by the control response creating a proportional response.

This study makes no assumptions regarding the particular significance of each early life stage response that was recorded. To characterize these biological responses in way that could be analyzed as teratogenic stress a conservative approach of un-rotated principal component analysis was applied. ZSCA response values were analyzed via principle components using JMP 9 (©2010 SAS institute). Four components were derived using pair-wise estimation to describe the variance of ELS endpoints. These components were tested as response variables in multiple regressions of sediment characteristics. The components were each tested in comparison to

public health indicators using a non-parametric risk analysis (regression using Weibull probability density function).

RESULTS

Chemical Contamination of Sediments in Lake Michigan Costal Streams

The scope and budget of this pilot project did not allow for full chemical screening. Contamination levels for 25 of the 48 sediment sample sites that were tested using the ZSCA are shown in Figure 4. Downtown/downstream Racine (ROOT_RAC1) was the most contaminated site at 1,098.71ng ∑PCB g^{-1} sediment, 4.23ng ∑Dioxin g^{-1} sediment, and 21.56ng ∑PBDE g^{-1} sediment. The least contaminated site was midstream Kewaunee (KEWR_KEWAN2) with: <MDL ∑PCB g^{-1} sediment, <MDL ∑Dioxin g^{-1} sediment, and 0.43ng ∑PBDE g^{-1} sediment. Compared to the amount of PCB and Dioxin observed in the sediments, PBDE contamination was relatively low. These results clearly demonstrate varying levels of chemical contamination (type and magnitude), with some sites/cities yielding highly contaminated sediment.

Total oxygen demand (TOD) was assessed in a subset of the sediment samples. During the oxygen demand tests, none of the sediments tested dropped below the critical limit of 2.0 mg L^{-1} (Hollert *et al.* 2003; Rocha *et al.* 2011; Strecker *et al.* 2011) within 72 hours (embryos for the current study were only exposed to sediment for 48hrs). None of the sediments that were tested for oxygen demand and also exhibited 100% mortality approached the lethal dissolved oxygen concentration of 0.88 mg L^{-1} during the 5 day TOD experiment. After 5 days two of the TOD-tested samples dropped to 1.7 mg L^{-1}, and 1.85 mg L^{-1} dissolved oxygen. These values are associated with growth retardation but not necessarily malformation or mortality (Braunbeck and Lammer 2006; Strecker *et al.* 2011). Nevertheless, those two samples did yield high levels of mortality and developmental retardation in the embryos.

Characterization of Early Life-Stage Endpoints Using Principal Component Analysis

The distributional properties of all ELS responses to sediment exposure at the 48 and 72 hour observation points are summarized in Tables 8A and 8B respectively. Principal components were calculated for these responses and biplots showing the relationships are presented in Figures 5 and 6. These components describe response patterns of malformations, mortality and developmental rate; the loading scores for these are displayed in Tables 9A and 9B.

At both time points, the first principal component (PC1) captured variance between mortality and occurrence of malformations (inverse correlations between death and occurrence of malformations is unsurprising). Both Figures 5A and 6A indicate that the variation between mortality and malformations produced a strong patterning effect within the data. At 48HPF, the second principal component (PC2) was strongly associated with higher levels of hypopigmentation, circulatory malformation, and heart malformations. This 48HPF PC2 was inversely associated with developmental rate and yolk edemas (Table 9A). Similar patterns were found at the 72HPF third principal component (PC3) in which higher PC3 scores were associated with more cranial and abdominal edema, and inversely associated with heart and circulatory malformation (Table 9B).

Early Life-Stage Response to Sediment Characteristics

Overall, significant positive correlations were found between TOD and all four of the 48 HPF principal components. TOD was also significantly correlated with PC 2, 3 and 4 at 72HPF (Table 10).

Using multiple linear regression analysis (standard least squares model) the four un-rotated ELS principal components (for both 48 and 72 HPF independently) were tested against the following sediment characteristics: percent organic content, RAPIDTOXKIT™ feeding inhibition

of pore water, and total ng g^{-1} of PCBs, Dioxins, and PBDEs. At 48HPF, the regression model yielded significant relationships between PC1 and all sediment characteristics except total PBDEs (Table 11A). This roughly describes a correlation between mortality and sediment contaminants in the first 48 hours of exposure. PC2 (48HPF) was significantly associated with percent organic content (Table 11B). PC3 (48HPF) was significantly associated with organic content and PCBs (Table 11C). No significant associations were found between the fourth principal component (48HPF) and the sediment characteristics (Table 11D). At 72HPF, significant associations were observed between PC1 and dioxins as well as pore water toxicity (Table 12A). PCBs, organic content, and pore water toxicity were significantly associated with PC2 at 72HPF (Table 12B). Organic content and total PCBs were significantly associated with PC3 (Table 12C) and PC4 (Table 12D) at 72HPF.

ELS Responses and Infant Health

Zebrafish ELS responses were tested for risk-relationships with infant health data from the corresponding communities using the non-parametric risk regression in JMP 9.0 (SAS 2010). Values for the infant health indicators are shown in Table 14. Aggregated infant mortality rate for the communities tested was about 8.4 per 1,000 births (range 2.8 to 14.2). This is notably higher than the overall state infant mortality rates for this same time period (6.5 from 2000 to 2009). Aggregated percent low birth weight for the communities was about 6.9% (range 3.8 to 9.7) which matches the overall state percentage of 6.9% from 2000-2009. Rates for aggregated CHD averaged about 9.7 per 1,000 births (range 0 to 20.6).

The 48HPF components did reveal one significant association with infant health (Table 13A). PC2 at 48 HPF was significantly associated (slower zebrafish development) with infant mortality. The 72HPF components showed significant risk-relationships with 3 infant health outcomes (Table 13B). PC1 at 72HPF was significantly associated (slower zebrafish development

and more malformations) with prevalence of infant mortality (Figure 7A) and PC3 at 72HPF was associated with prevalence of congenital heart disease (Figure 7B). There was also a significant relationship between low birth-weight and the 72 HPF PC4 (Table 13B). Table 14 displays the data used to calculate infant mortality, CHD prevalence, and percent low birth weight.

DISCUSSION

Multivariate models revealed weak, but significant, associations between ZSCA responses and chemical contaminants, organic content, and pore water toxicity. This supports the notion that ZSCA responds to sediment and watershed characteristics near human populations. The low R^2 values of the models suggest that factors, perhaps contaminants not measured in this study, could be responsible for the teratogenic responses characterized by the principal component analysis. Both ∑PCB and ∑Dioxin showed significant associations with the teratogenic response of zebrafish embryos both at 48 and 72 HPF. Contaminant levels of ∑polybrominated diphenyl ethers from the sediments of these streams (all of which empty to Lake Michigan) were sparse, and did not seem to affect the ZSCA results. BDE-209 was the most frequently observed congener of PBDE in these samples which corroborates for Great Lakes streams what is suggested by previous studies on Great Lakes sediments (Song *et al.* 2005; Zhu and Hites 2005).

RAPIDTOXKIT™ inhibition was able to significantly describe some of the variance from the first ELS principal components at both 48hpf and 72hpf. Other studies have identified the RAPIDTOXKIT™ assay as an attractive tool for rapid, cost-effective screening of 'new' pollutants such as drugs which may threaten the biological communities of the aquatic environment (Nalecz-jawecki and Persoone 2006; Torokne *et al.* 2007). When applied to this pilot project, the use of this high throughput assay allowed for an investigation of potential stressors that are not included in the chemical analysis.

A high tolerance for anoxic conditions increases the appeal of using zebrafish as model organisms for toxicity testing of native (unaltered) sediments. The fishes' tendency to "endure" stressful conditions allows researchers to observe subtle, non-lethal endpoints such as abnormalities in circulation and cardio-vascular development. The principal components analysis allowed for a generalized characterization of teratogenic response without the potential bias of selecting individual endpoints to investigate or the need to categorize observations into "lethal or non-lethal" to create mortality-based metrics. In this study a relationship was observed between the PC3 (which represents cardiovascular development and some edemas) at 72HPF and human health as well as chemical contaminants. Such an observation using native sediments may not be possible in organisms more sensitive to anoxic conditions or if the observed malformations were categorized into metrics.

Strecker et al. (2011) observed that a 72 hour pre-test incubation of sediments in the plate wells can be used to establish minimal levels of oxygen even above the sediment surface. In Zebrafish embryo contact tests with native sediments from Lake Skadar (Montenegro), oxygen concentrations rapidly decreased upon addition of the sediments to levels as low as 0.8 mg/L, but recovered to 2.5 and 3.5 mg/L after 72 and 144 h, respectively (Strecker *et al.* 2011). These observations suggested that, during the initial mixing procedure, dissolved oxygen is rapidly used up by inorganic and organic redox processes; upon stabilization of the sediment surface, however, albeit low, but sufficient oxygen conditions are re-established with time. In sediments from the current study, TOD was significantly correlated with ELS responses (more so at 48HPF than 72HPF). It is possible that other factors leading to increased oxygen demand also lead to teratogenic response. Also, oxygen demand in sediments can alter the bioavailability of toxic substances such as Mercury compounds (Taylor *et al.* 2012) and other metals (De Jonge *et al.* 2012). The protocol used in the present study does not allow us to make the distinction

between oxygen stress and response to pollution. However, the results seem to suggest a stronger influence of hypoxia during the 48 hour exposure period. Whereas the 72 HPF observations (which had higher dissolved oxygen levels), after 24 hours of development in clean water, were more informative when compared to watershed contamination conditions and public health metrics.

Implications of ELS Endpoint Response to City-Bound Watersheds

The literature supports ZSCA as a useful tool to sensitively detect toxic effects of sediments and exhibits high ecological relevance (since it accounts for bioavailability of contaminants) (Hollert *et al.* 2003; Keiter *et al.* 2009; Keiter *et al.* 2010; Kosmehl *et al.* 2006; Rocha *et al.* 2011; Strecker *et al.* 2011). My results confirm that ZSCA yields biologically-meaningful data regarding the toxic response of zebrafish embryos to environmental samples. Furthermore, these responses were significantly associated with watershed and sediment conditions across multiple human-inhabited boundaries along Lake Michigan. Whereas previous studies using fish teratogenicity have focused on targeted watersheds to investigate expected environmental perturbation (Hollert *et al.* 2003; Keiter *et al.* 2009; Keiter *et al.* 2010; Kosmehl *et al.* 2006; Rocha *et al.* 2011; Strecker *et al.* 2011); the current study demonstrates that this technique could also serve as a toxic screen for human communities with uncharacterized environmental health.

The availability of the Wisconsin Pediatric Cardiac Registry presented an opportunity to investigate a human health outcome with suspected geographically-linked risk factors (Cedergren *et al.* 2002; Cronk *et al.* 2011), suggesting that CHD occurrence, relative to other human health indicators, may be sensitive to environmental stress. While CHDs are the most common birth defects, they are still relatively rare (Table 14). This can hinder the confidence that rates calculated for small geographic areas reflect meaningful differences among

communities. This issue was considered and the cities with potentially suspicious birth-prevalence were noted in JMP 9.0 (©2010 SAS institute). I observed that the lesser-populated cities did not occupy the extreme values describing the probability distribution displayed in Figures 7A and 7B. For this exploratory study, it was not necessary to assume specific causal pathways between distinct diagnoses of CHD and teratogenic stress. Therefore, all diagnoses of CHD were included for analysis and were aggregated across ten years. By broadening the scope of CHD records, we were able to minimize stochastic effects on the calculated CHD prevalence. Similar considerations are true of infant mortality.

There may be geographic misclassification of congenital heart defect occurrence due to maternal mobility during pregnancy. Many studies, however, suggest limited exposure misclassification due to residential mobility (Canfield *et al.* 2006; L Chen *et al.* 2010; Fell *et al.* 2004; Krieger *et al.* 2002; Lupo *et al.* 2010; Shaw and Malcoe 1992). Geographic misclassification of the three other infant health indicators is also possible. Addresses are self reported on the birth record, and subject to various errors (use of mailing address and not residence, misspelling of town or city names etc.). However, this should present limited, if any impact, on the findings.

The comparison of CHD to sediment teratogenicity yielded suggestive results to support the hypothesis, as we found significant correlations between PC3 and the prevalence of congenital heart disease. Zebrafish have been utilized to elucidate underlying mechanisms of cardiac development and human congenital heart diseases, as well as potential pathways that may modulate cardiac regeneration (Bakkers 2011; Heideman *et al.* 2005; Tu and Chi 2012). It therefore stands to reason that, despite obvious structural differences between fish and mammal hearts, PC3 could indicate a response that is analogous to pathways producing human CHD. We cannot speculate, however, on what the specific molecular mechanisms or exposure pathways may be for the current study.

The relationships observed between vital statistics and principal components are unsurprising. Previous work supports the notion that these indicators in Wisconsin are closely related to social determinants of health (M Dellinger *et al.* 2012; Robert and Booske 2011; Vila *et al.* 2007; WDHS 2012b; Webb *et al.* 2011). The design of the current study did not accommodate controlling for social determinants of health. Nevertheless, meaningful interpretation is possible. Given the trends observed in previous work with WI vital statistics (M Dellinger *et al.* 2012) it is likely that geographic areas with higher health disparities are likely to contain features that would increase stream toxicity. Certain ZIP codes in Milwaukee, for example, contain socially-disadvantaged neighborhoods along-side streams which receive considerable urban runoff. The impoverished nature of these areas, both urban and rural, was evident at the time of sediment collection.

ZSCA at the current level of development, does not inform researchers to the presence of specific contaminates or stressors in the environment. This application of the assay is non-specific, but is so by design. As previously discussed (Dellinger *et al.* 2011; M Dellinger *et al.* 2012) toxicology, epidemiology, and risk assessment research face an insurmountable volume of potential toxic agents mixed together within dynamic biological, political, and geographical systems. This technique allows for analysis and interpretation of toxicological patterns which provide the necessary indicators to monitor environmental health.

Integrative Toxicity Screening Using EDA

This work demonstrates the use of bioassays to characterize teratogenic (toxic) response to stream sediments in a way compatible with probabilistic risk modeling. These metrics were then successfully applied to an assessment of sediment toxicity (using chemical analysis) and risk assessment (using public health data). The latter application should be treated

with cautious optimism. The hypothesis that significant risk-relationships would exist between public health patterns and effect-directed assessment is supported.

Interpretation of the principal components and the risk analysis indicate an increased risk of historic infant mortality in cities with streams yielding higher levels of ELS malformations and slower development at both observation times. This included developmental delays, edemas, and structural malformations. PC2 at 48HPF and PC1 at 72HPf may describe a generalized stress-response that responds to stressed streams in communities of low socio-economic status. Furthermore (and perhaps most intriguing) principal components describing variance in heart malformations and some edemas at 72HPF were significantly associated with increased incidences of congenital heart disease in humans. Though, structurally distinct from human hearts, zebrafish cardiogenesis is often proposed as a useful model for understanding human cardiovascular development (Bakkers 2011; Heideman *et al.* 2005; Tu and Chi 2012). These results support the notion that common stressors, acting upon streams and humans on a regional scale, induce teratogenic effects. Therefore, ZSCA appears to be a good candidate for quantifying environmental perturbation that may be relevant to human populations.

Whether or not this is due to common risk factors between human exposures and stream exposure remains a matter of speculation. Screening tools should not be held to the expectation of representing an exposure assessment. Nor do I suggest EDA can replace exposure assessment. Nevertheless, similar responses between the sentinel or screening populations and human populations bolster the idea that environmental screening tools yield toxicologically meaningful information for humans.

Ecological risk assessors face increasing demands to assess more chemicals, with greater speed and accuracy, and to do so using fewer resources and experimental animals. Furthermore, new approaches in biological and computational sciences should be applied to

meet these challenges (Ankley *et al.* 2010). Reduction of cost and time for testing, broader coverage of chemicals and their mixtures, reduction of animal suffering, increased integration of toxicologic and population-based data remain priority areas for advancing risk assessment (NRC 2007).

This pilot study was conducted to investigate the use of novel tools for risk assessment that could benefit children's health specifically. Future efforts should broaden the scope of applying bioassays to investigate the environmental hazards to human populations. Larger projects could include: more comprehensive watershed sampling, integration of other EDAs (mutigenicity, cytotoxicity, *etc.*), identification of within population variability and confounder variables, and broader geographic range. Biologically directed fractionation techniques using semi-permeable membrane devices (SPMD), for example, are demonstrated to adequately represent sediment contamination in toxicity testing with multiple organisms, including fish (Heinis *et al.* 2004). Using SPMDs as a medium to test environmental contamination with ZSCA presents an interesting possibility for future study and could further reduce concerns of hypoxia affecting the embryos.

EDA bioassays normally used for ecological screening can be useful as indicators of environmental stress to humans, and have the potential to expand understanding of linked environmental risks to ecological and human health. Much work remains before sediment contact assays and other EDA techniques can be used to guide risk management or assessment. However, the patterns of teratogenic responses demonstrated here suggest that using information regarding the type and degree of toxicity could be assessed absent of chemical screening. Further calibration (i.e. identifying key embryo malformations for metric calculation) of this technique could allow for the prioritization of chemical screening, which would also increase the efficiency of biomonitoring efforts.

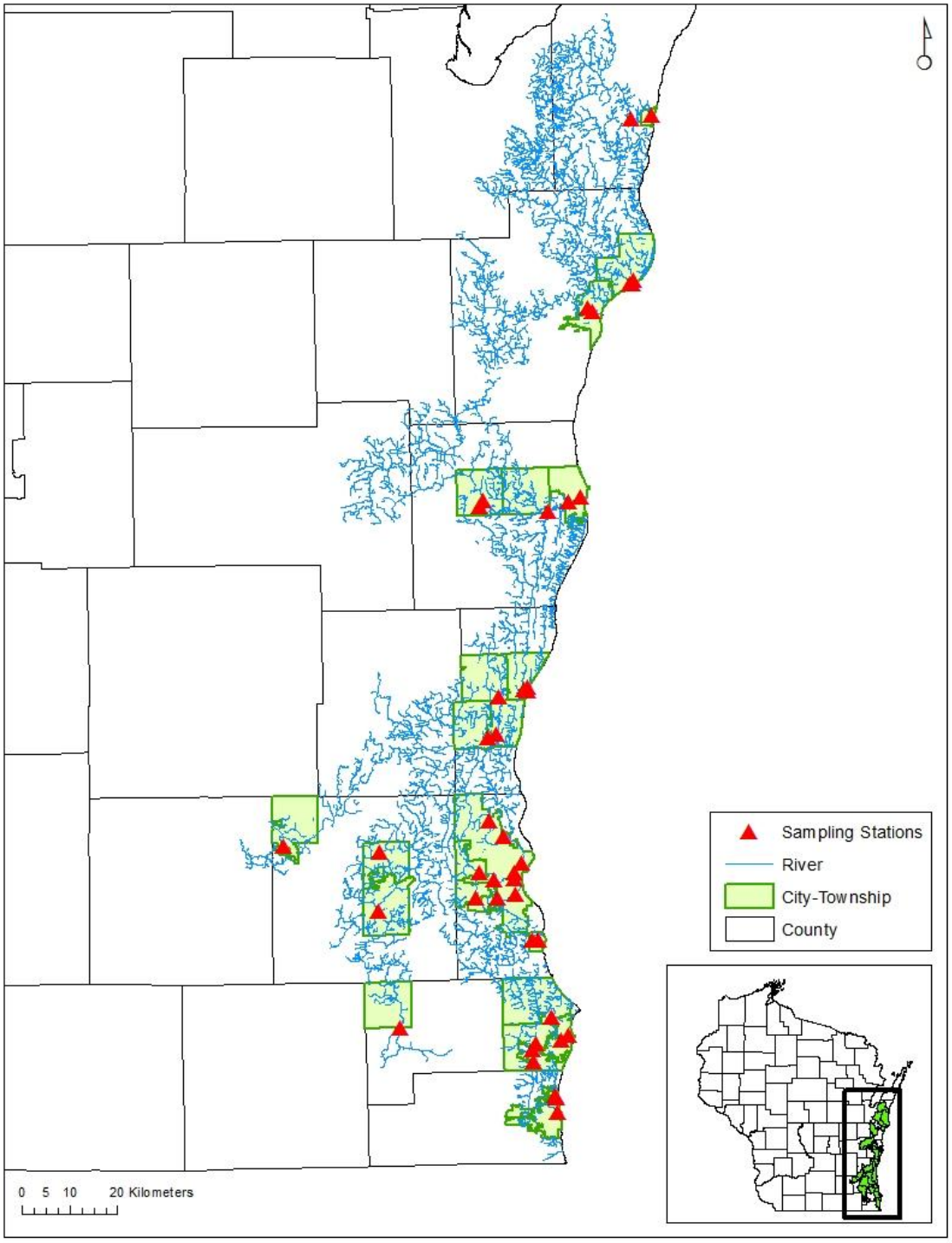


Figure 3: Sites (N=48) for sediment sampling of streams representing 26 human populations along Lake Michigan in Wisconsin. City boundaries are represented in green, sediment sampling locations are represented by red triangles, water bodies are displayed in blue to provide context.

Table 7: Data requested from the Wisconsin Pediatric Cardiac Registry and the Wisconsin Department of Health Services. All birth counts were aggregated over ten years (2000-2009) by address of mother at time of birth.

Birth Outcome Data Requests	
Infant Health Outcome	**Birth Count 2000-2009**
Congenital Heart Disease	Total Diagnoses
Infant Deaths	Total Deaths, age < 1 year
Premature Births	Births 30-37 Weeks Gestational Age
Low Birth Weight	Births < 2499 g Weight
Total	All births

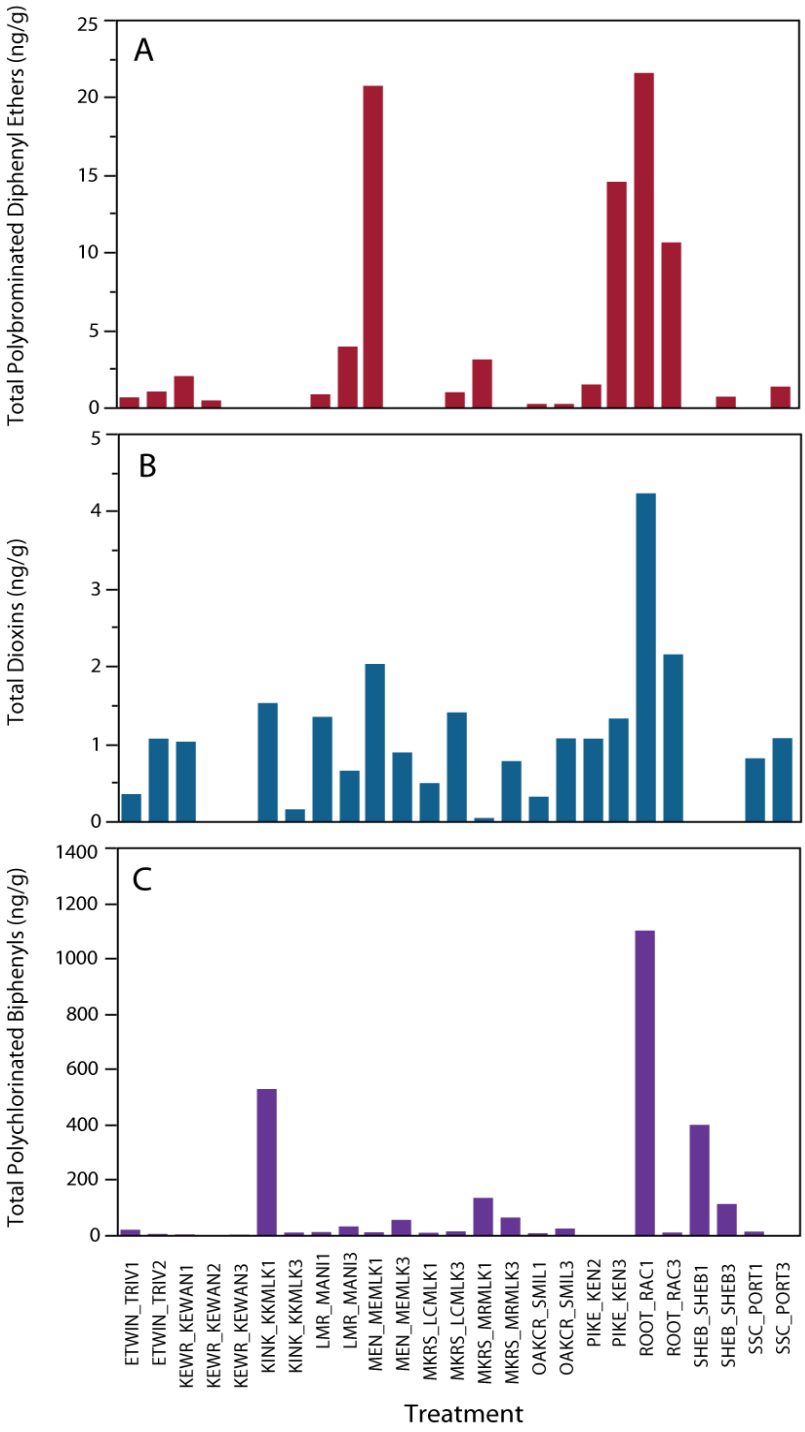


Figure 4: Chemical contaminates (nanograms) per gram sediment from 10 cities and 4 Milwaukee rivers, totaling 25 samples (some upstream, some downstream). Y axis represents ∑PBDE (A), ∑Dioxin (B), and ∑PCB (B) in ng/g. The x-axis lists sample codes which represent the watershed then city respectively, 1's represent furthest downstream (relative to city boundaries) 3's represent furthest upstream (relative to city boundaries) 2's represent sites that were either from equally contributing, but separate, watersheds or midstream sites.

Table 8: Treatment summary statistics of early life-stage endpoints: death, hatch, development, lack of pigmentation, cardiac edema, yolk edma, cranial edema, abdominal edema, malformation of body axis, somite development, tail malformation, eye malformation, heart/heartbeat malformation, circulatory malformation, and head malformation at 48 (A) and 72 (B) hours post-fertilization. Replicates consisted of 6 embryos yielding a maximum possible response of 6.

A. Early Life-stage Endpoints - 48 hours

48HPF ELSE	Mean	Median	Std Dev	Max
Dead	2.311	2	1.91	6
Unhatched	3.69	4	1.91	6
Underdeveloped	2.69	3	1.83	6
HypoPigment	0.51	0	1.09	5
Ed:Cardiac	0.04	0	0.22	2
Ed:Yolk	1.03	0	1.45	6
Ed:Cranial	0	0	0	0
Ed:Abdom	0.05	0	0.27	3
Mal:Axis	0.07	0	0.29	2
Mal:Somite	1.83	2	1.63	5
Mal:Tail	0.12	0	0.41	4
Mal:Eyes	0.06	0	0.25	2
Mal:Heart	0.23	0	0.66	4
Mal:Circ	0.09	0	0.41	4
Mal:Head	0.06	0	0.26	2

B. Early Life-stage Endpoints - 72 hours

72HPF ELSE	Mean	Median	Std Dev	Max
Dead	2.65	2	2.05	6
Unhatched	3.03	3	1.97	6
Underdeveloped	2.01	2	1.77	6
HypoPigment	0.81	0	1.28	5
Ed:Cardiac	0.56	0	0.92	5
Ed:Yolk	0.89	0	1.21	5
Ed:Cranial	0.04	0	0.23	2
Ed:Abdom	0.06	0	0.32	3
Mal:Axis	0.53	0	0.80	4
Mal:Somite	0.41	0	0.84	5
Mal:Tail	0.40	0	0.74	3
Mal:Eyes	0.20	0	0.55	3
Mal:Heart	0.94	0	1.24	5
Mal:Circ	1.14	1	1.29	5
Mal:Head	0.26	0	0.64	3

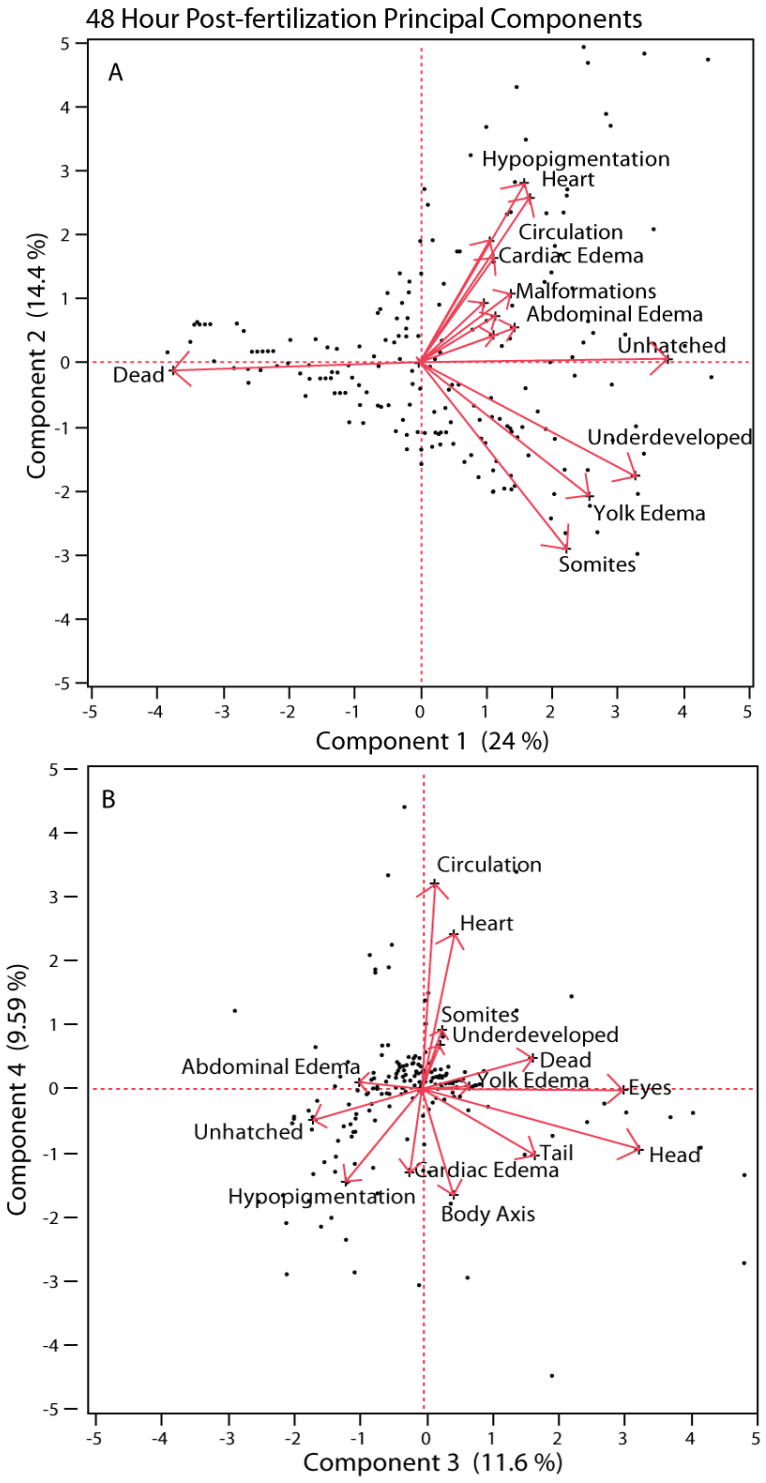


Figure 5: Biblot (a matrix of scatterplots of the scores for pairs of principal components overlaid with a matrix of two-dimensional representations of factor loading) of early life-stage endpoints recorded at 48 hours post-fertilization. X-axis represents component 1 (A) and component 3 (B) Y-axis represents component 2 (A) and component 4 (B). Labeled biplot rays show eigenvectors of endpoints relative to each component in multivariate space.

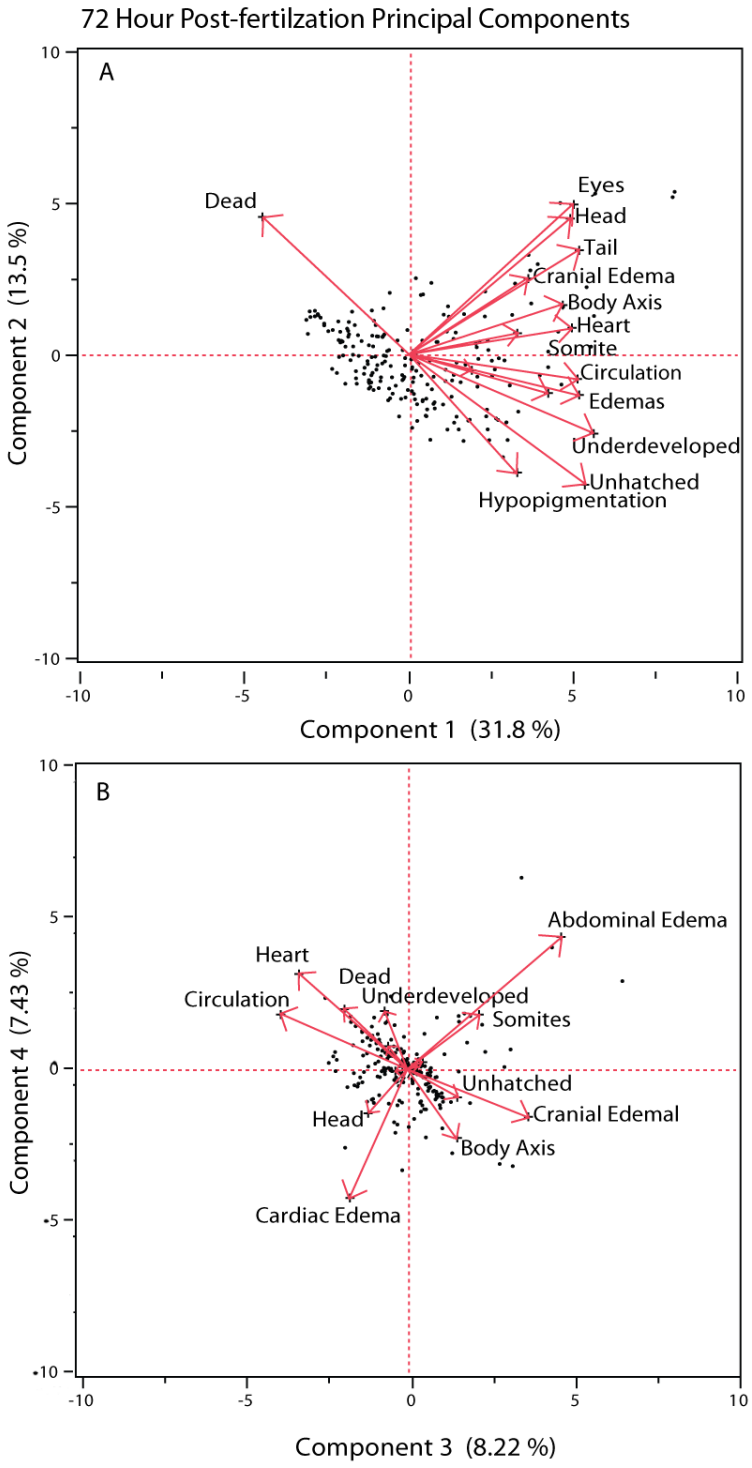


Figure 6: Biblot (a matrix of scatterplots of the scores for pairs of principal components overlaid with a matrix of two-dimensional representations of factor loading) of early life-stage endpoints recorded at 72 hours post-fertilization. X-axis represents component 1 (A) and component 3 (B) Y-axis represents component 2 (A) and component 4 (B). Labeled biplot rays show eigenvectors of endpoints relative to each component in multivariate space.

Table 9: Loading scores for principal components of 48 hour post-fertilization (A) and 72 hour post-fertilization (B) early life-stage endpoints. Dominant loading scores are in bold.

A. Loading Scores for Principal Components - 48 hours

48HPF ELSE	Prin1	Prin2	Prin3	Prin4
Dead	**-0.85**	-0.03	0.38	0.11
Unhatched	**0.85**	0.01	-0.37	-0.11
Underdeveloped	**0.74**	**-0.40**	0.06	0.15
Hypopigment	0.36	**0.63**	-0.26	-0.33
Ed:Cardiac	0.25	0.37	-0.04	-0.30
Ed:Yolk	**0.58**	**-0.47**	0.16	0.01
Ed:Cranial	0	0	0	0
Ed:Abdom	0.22	0.21	-0.21	0.02
Mal:Axis	0.26	0.16	0.11	-0.37
Mal:Somite	0.50	**-0.66**	0.07	0.21
Mal:Tail	0.33	0.12	0.39	-0.23
Mal:Eyes	0.31	0.24	**0.69**	-0.004
Mal:Heart	0.38	**0.58**	0.11	**0.55**
Mal:Circ	0.24	**0.43**	0.05	**0.72**
Mal:Head	0.26	0.09	**0.74**	-0.21

B. Loading Scores for Principal Components - 72 hours

72HPF ELSE	Prin1	Prin2	Prin3	Prin4
Dead	**-0.55**	**0.56**	-0.24	0.25
Unhatched	**0.66**	**-0.53**	0.19	-0.11
Underdeve	**0.69**	-0.32	-0.08	0.24
Hypopigment	**0.40**	**-0.49**	-0.04	-0.01
Ed:Cardiac	**0.52**	-0.16	-0.22	**-0.53**
Ed:Yolk	**0.64**	-0.17	-0.07	0.09
Ed:Cranial	**0.45**	0.31	**0.46**	-0.20
Ed:Abdom	0.24	-0.06	**0.58**	**0.54**
Mal:Axis	**0.58**	0.20	0.19	-0.28
Mal:Somite	**0.41**	0.09	0.27	0.22
Mal:Tail	**0.64**	**0.43**	0.06	0.03
Mal:Eyes	**0.62**	0.61	0.04	0.01
Mal:Heart	**0.61**	0.12	**-0.41**	0.39
Mal:Circ	**0.64**	-0.10	**-0.48**	0.23
Mal:Head	**0.61**	**0.56**	-0.14	-0.18

Table 10: Properties of standard linear regression model for total oxygen demand vs. principal components of early life-stage endpoints.

Oxygen Demand vs. Zebrafish Response	
Component	r^2
Prin1 (48HPF)	**0.088***
Prin2 (48HPF)	**0.183***
Prin3 (48HPF)	**0.208***
Prin4 (48HPF)	**0.117***
Prin1 (72HPF)	0.002
Prin2 (72HPF)	**0.136***
Prin3 (72HPF)	**0.181***
Prin4 (72HPF)	**0.153***

**p<0.05*

Table 11: Multiple linear regression model (standard least squares) effects for 48 hour post-fertilization principle components 1(A), 2(B), 3(C) and 4(D). Model parameters are total PBDEs congeners, total PCB congeners, total Dioxins (all log10 (ng g^{-1} sediment)) as well as inhibition (proportional feeding inhibition of *T. platyurus* larvae), and percent organic content by weight (arcsine transformed). T Ratio is the ratio of the estimate to its standard error.

A. PC1 Model (R^2=0.47, P=0.0001)

Prin1 (48HPF)	Estimate	t Ratio
% Organic*	**-4.80**	**-3.35**
∑PCB ng/g*	**-1.07**	**-5.59**
∑Dioxin ng/g*	**-3.97**	**-3.72**
∑PBDE ng/g	-0.33	-0.79
Inhibition*	**-1.83**	**-3.32**

B. PC2 Model (R^2=0.14, P=0.0051)

Prin2 (48HPF)	Estimate	t Ratio
% Organic*	**-4.62**	**-3.23**
∑PCB ng/g	-0.32	-1.67
∑Dioxin ng/g	1.36	1.28
∑PBDE ng/g	0.10	0.23
Inhibition	-0.12	-0.21

C. PC3 Model (R^2=0.149, P=0.0034)

Prin3 (48HPF)	Estimate	t Ratio
% Organic*	**3.25**	**2.29**
∑PCB ng/g*	**0.60**	**3.15**
∑Dioxin ng/g*	**-0.54**	**-0.51**
∑PBDE ng/g	0.03	0.08
Inhibition	-0.41	-0.74

D. PC4 Model (R^2=0.04, P=0.539)

Prin4 (48HPF)	Estimate	t Ratio
% Organic	2.34	1.84
∑PCB ng/g	-0.02	-0.14
∑Dioxin ng/g	-0.25	-0.27
∑PBDE ng/g	-0.19	-0.51
Inhibition	0.12	0.24

**p<0.05*

Table 12: Multiple linear regression model (standard least squares) effects for 72 hour post-fertilization principle components 1(A), 2(B), 3(C) and 4(D). Model parameters are total PBDEs congeners, total PCB congeners, total Dioxins (all log10 (ng g^{-1} sediment)) as well as inhibition (proportional feeding inhibition of *T. platyurus* larvae), and percent organic content by weight (arcsine transformed). T Ratio is the ratio of the estimate to its standard error.

A. PC1 Model (R^2=0.13, P=0.009)

Prin1 (72HPF)	Estimate	t Ratio
% Organic	1.33	0.68
∑PCB ng/g	-0.49	-1.87
∑Dioxin ng/g*	**-2.93**	**-2.03**
∑PBDE ng/g	-0.95	-1.7
Inhibition*	**-1.70**	**-2.28**

B. PC2 Model (R^2=0.28, P=0.0001)

Prin2 (72HPF)	Estimate	t Ratio
% Organic*	**3.48**	**2.84**
∑PCB ng/g*	**0.61**	**3.75**
∑Dioxin ng/g	0.16	0.17
∑PBDE ng/g	0.59	1.67
Inhibition*	**1.07**	**2.27**

C. PC3 Model (R^2=0.19, P=0.0003)

Prin3 (72HPF)	Estimate	t Ratio
% Organic*	**-3.04**	**-3.49**
∑PCB ng/g*	**-0.24**	**-2.07**
∑Dioxin ng/g	0.10	0.16
∑PBDE ng/g	-0.18	-0.72
Inhibition	-0.31	-0.92

D. PC4 Model (R^2=0.21, P=0.0001)

Prin4 (72HPF)	Estimate	t Ratio
% Organic*	**2.45**	**2.68**
∑PCB ng/g*	**0.31**	**2.54**
∑Dioxin ng/g	0.92	1.35
∑PBDE ng/g	0.21	0.79
Inhibition	0.49	1.4

**p<0.05*

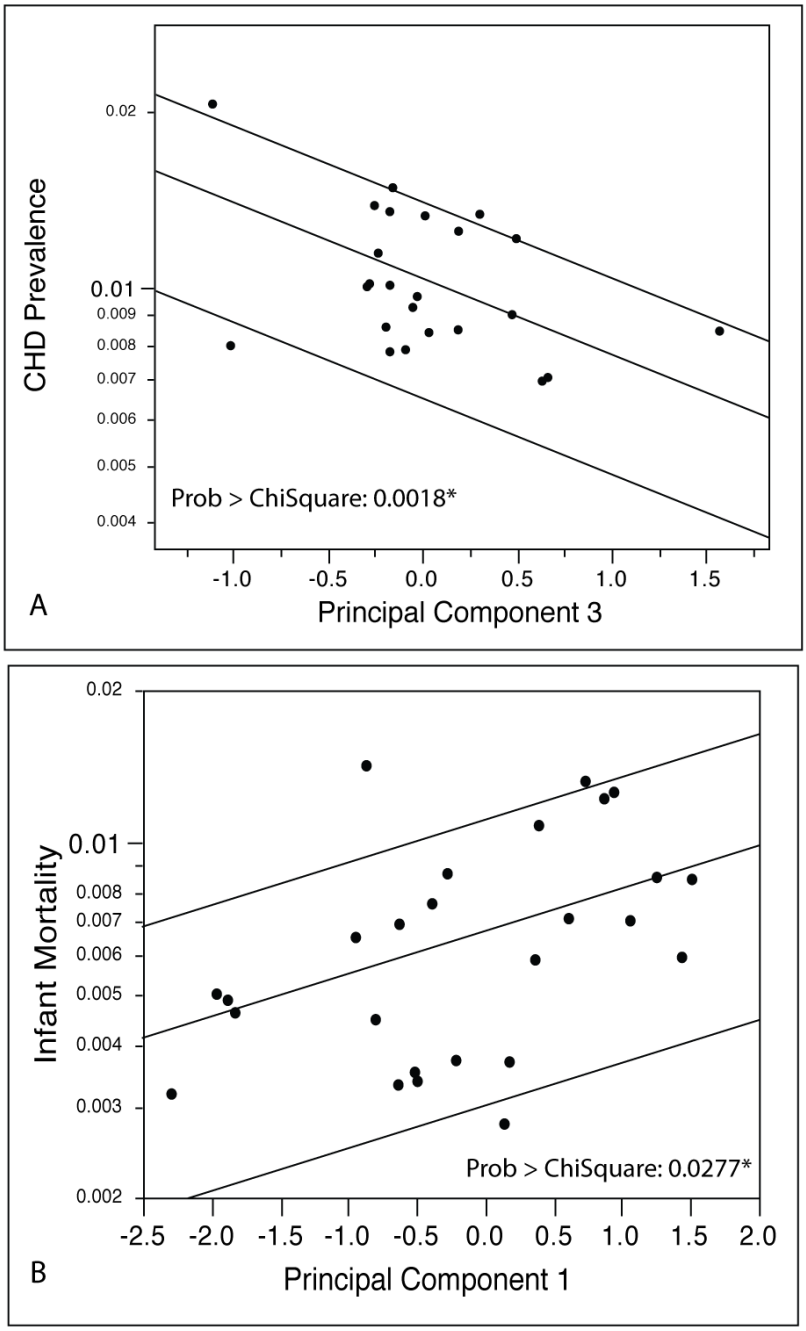


Figure 7: Risk-regression of 72 hours post fertilization principal component 3 and congenital heart disease (A); and principal component 1 and infant mortality (B). Prevalences are calculated as incidence of death within first year of life, or diagnosis of congenital heart disease, divided by total births for that time period in the corresponding city or zipcode. Lines represent the 10th, 50th, and 90th percentiles of the fitted relationship.

Table 13: Effect likelihood ratio tests for risk models comparing mean principal component scores of early life-stage endpoints to birth prevalence of vital statistic outcomes (low birth weight , premature births, and infant mortality rates) and birth prevalence of congenital heart disease (all diagnoses).

A: Effect Likelihood Tests: 48 HPF Zebrafish Responses and Birth Outcomes

Birth Outcome	Principal Component	Chi-Squared	Probability > Chi-Squared
Premature Birth	PC1	0.083	0.773
	PC2	2.857	0.091
	PC3	0.392	0.531
	PC4	0.454	0.500
Low Birth-Weight	PC1	0.354	0.552
	PC2	2.124	0.145
	PC3	0.188	0.665
	PC4	0.717	0.397
Infant Mortality	PC1	2.991	0.084
	PC2	**4.534**	**0.032***
	PC3	0.272	0.602
	PC4	0.003	0.951
Congenital Heart Disease	PC1	0.027	0.870
	PC2	1.301	0.254
	PC3	0.058	0.810
	PC4	0.396	0.529

B: Effect Likelihood Tests: 72 HPF Zebrafish Responses and Birth Outcomes

Birth Outcome	Principal Component	Chi-Squared	Probability > Chi-Squared
Premature Birth	PC1	0.104	0.747
	PC2	0.044	0.833
	PC3	2.004	0.157
	PC4	1.047	0.306
Low Birth-Weight	PC1	0.782	0.377
	PC2	0.112	0.738
	PC3	1.245	0.265
	PC4	**4.200**	**0.043***
Infant Mortality	**PC1**	**4.845**	**0.028***
	PC2	0.859	0.354
	PC3	0.753	0.385
	PC4	1.002	0.317
Congenital Heart Disease	PC1	0.269	0.604
	PC2	0.216	0.642
	PC3	**9.714**	**0.002***
	PC4	0.141	0.708

*P<0.05

Table 14: Data used to calculate indicators which showed significant increased risk in association with stream teratogenicity. Data were queried from the Wisconsin Department of Health Services and The Wisconsin Pediatric Cardiac Registry. All birth counts were aggregated over ten years (2000-2009) by address of mother at time of birth.

*Rates are calculated per 1,000 births.

City/ZIP	Total Births	Infant Deaths	IMR*	CHD cases	CHDR*	Low Birth Weight	%LBW
Kewaunee	337	2	5.93	5	14.84	16	4.75
Sturtevant	602	2	3.32	8	13.29	40	6.64
Mount Pleasant	716	9	12.57	0	0.00	51	7.12
Saukville	719	2	2.78	5	6.95	27	3.76
Sheboygan Falls	941	3	3.19	8	8.50	49	5.21
Two Rivers	1284	9	7.01	13	10.12	87	6.78
Waterford	1301	6	4.61	18	13.84	78	6.00
Plymouth	1342	6	4.47	13	9.69	60	4.47
Port Washington	1421	5	3.52	19	13.37	69	4.86
Pewaukee	1554	11	7.08	32	20.59	87	5.60
Grafton	1616	6	3.71	15	9.28	63	3.90
Cedarburg	1627	6	3.69	13	7.99	64	3.93
Caledonia	1757	15	8.54	4	2.28	90	5.12
South Milwaukee	2304	20	8.68	28	12.15	130	5.64
Oconomowoc	2961	10	3.38	40	13.51	172	5.81
Manitowoc	4358	33	7.57	34	7.80	272	6.24
Wauwatosa	5529	27	4.88	39	7.05	281	5.08
West Allis	6974	59	8.46	59	8.46	434	6.22
Sheboygan	8263	57	6.90	71	8.59	423	5.12
53203,08,33	8741	124	14.19	88	10.07	850	9.72
53202,11,12	9664	118	12.21	76	7.86	815	8.43
Waukesha	11749	59	5.02	147	12.51	710	6.04
Kenosha	14829	87	5.87	151	10.18	1004	6.77
Racine	16287	176	10.81	187	11.48	1235	7.58
53209,18,23	19378	256	13.21	163	8.41	1764	9.10
53207,15,19	22167	144	6.50	200	9.02	1319	5.95
Overall	148421	1252	8.44	1436	9.68	10190	6.87

CHAPTER 3: USING PREDICTIVE ANALYTICS TO GUIDE TOXICOLOGICAL RISK ASSESSMENT AND INDICATOR PRIORITIZATION

INTRODUCTION

Employing Risk Propagation and Predictive Models

As discussed in Chapter 1, there is little consensus regarding the potential health effects (or Hazard Identification) of many chemicals. This is true with CECs, but even the effects of legacy contaminants such as Mercury (Hg) and Polychlorinated Biphenyls (PCBs) continue to be scrutinized within the context of interacting biological, political, and geographic systems (JA Dellinger *et al.* 2012b). The chemical-specific nature in which risk assessment is traditionally carried out limits the ability to address mixtures, interactions, unknowns and volume of stressors (which could include non-chemical factors). In light of these challenges, of managing public health in the Great Lakes and other regions will likely persist and grow. Novel solutions are needed to deal with complexity regarding human health and pollution.

Large-scale human longitudinal studies are rare – see for example the National Children's Study that will follow 100,000 women and their children from before conception to early adulthood (Branum *et al.* 2003; Hirschfeld *et al.* 2010). The success of such studies, designed to produce sufficient power to evaluate multiple interacting environmental exposures, depends on the accurate, cost-effective, characterization of environmental exposures assumed to be related to the health outcomes of interest (Strauss et al., 2010). However, seldom are there sufficient resources available to unambiguously demonstrate cause-effect relationships between environmental stressors and health outcomes. The challenge of deciding how and what to measure is further complicated for studies where constraints preclude the collection of all necessary measurements, especially in developing nations (Nriagu et al., 2010, UNDP 2006).

Calls to better understand how the environment can affect public health have resulted in governmental actions. For example, in 2002 the U.S. Congress allocated funding to the

Centers for Disease Control and Prevention (CDC) to develop the National Environmental Public Health Tracking Program which will "provide information communities can use to improve their health" (CDC, 2004). This resulted requests for development of indicators of environmental risk to human health (CEC, 2006, CDC, 2006, EPA, 2003a). In most developed countries, health departments collect vital health data that can be aggregated by counties, regions, and sometimes city or postal code levels. These data are meant to assist policy makers and researchers in tracking human health and investigating relationships between human health and independent factors. However correlational relationships based on these data seldom carry sufficient weight to influence policy development (Wakefield 2008; Wakefield and Haneuse 2008).

Many epidemiological studies demonstrate correlations between environmental stressors and human health endpoints (Wilhelm and Ritz, 2004, Wilhelm et al., 2007, Wigle et al., 2008, Yauck, 2003) while others investigate relationships between environmental stressors and ecological conditions (Wang et al., 2010, Alberti et al., 2007). There is increasing interest in integrating these two disciplines. By considering humans and ecosystems as part of an integrated system exposed to common environmental conditions, integrated risk assessment can help to better protect both human and non-human populations (EPA 2003; Suter and Cormier 2008; Suter *et al.* 2003). In this chapter, I present a framework for integrating the fields of ecological and human health risk assessment using examples from Wisconsin, USA and the Dobrogea regions of Romania.

Data Patterning and Predictive Modeling Approach

Chemical, physical, or biological agents in the natural environment may exert a combined effect on organisms, interfere with one another, or interact to create a larger overall effect. To understand these relationships, multivariate stochastic methods have been used to

model individual risks and total combined risks to aquatic communities (Bedoya et al. 2011; Novotny et al. 2005; Novotny et al. 2001). These models are based on statistical characteristics of randomness, cross-correlation, and autocorrelation in which probabilities, random fluctuations, deterministic relationships and time series are considered. As a systems-based approach, Ecological Risk Assessment (ERA) must account for this. Failing to account for interactions of multiple environmental influences on the health of human populations is to ignore the fact that humans are part of an ecosystem.

Basara and Yaun (2008) used Self Organizing Maps (SOMs) to investigate the hypothesis that communities with similar environmental characteristics exhibit similar distributions of disease. By clustering communities of New York counties based on ninety-two environmental variables, they reported a positive relationship between environmental conditions and community health outcomes. Self-organizing maps, a neural network form of clustering, use an algorithm which is tolerant of nonlinear and nonparametric data (Kohonen, 2001). Using this approach helps researchers to overcome challenges of non-linearity and skewed data distributions that have limited past efforts (Basara and Yuan, 2008).

A variety of disciplines have embraced the use of artificial intelligence models or artificial neural networks (ANN). In financial literature, neural network methods and hybrid models perform better compared with traditional methods for managing and evaluating financial services such as credit risk evaluation, bankruptcy prediction, and financial forecasting (Bahrammirzaee, 2010). Also, the prediction of SOM cluster identification based on biotic integrity scores using ANNs has been successfully applied to stream ecosystems (Novotny et al., 2005, Novotny et al., 2009). The development of similar predictive models using human health data (such as vital statistic indicators or medical biomarkers) could allow efficient and robust

identification of key management goals and prospective identification of hazards for risk assessment.

Predictive analytic approaches, which employ data patterning artificial intelligence learning, may help to draw meaningful analysis from the complex systems that encompass environmental public health. The application of unsupervised learning algorithms to public health data provides unique patterns of outcome variables relating to human populations. SOM analysis (Kohonen 2001), and other neural net algorithms, is a learning process. The version implemented here using JMP 9 (©2010 SAS institute), is a simple variation on k-means clustering. This technique produces clusters of data that are near each other in multivariate space. The SOM technique does this without reliance on assumptions of linear relationships or parametric data.

The goal of this chapter is to demonstrate a predictive analytic approach to risk assessment using unsupervised and supervised learning models. Using unsupervised learning, clusters of Wisconsin counties with similar multivariate health indicator patterns (measured through vital statistic indicators from the years 2002-2006) and from Dobrogea, Romania civil divisions (calculated through vital statistical indicators from 2007-2010) were identified. Secondly, this design applies supervised learning to predict the relationships between these health patterns and predictive factors of interest. I hypothesize that this process can be used to evaluate the predictive potential of models built from diverse sets of predictor variables and independent variables characterized by health patterns. The predictive value from these models can be used to infer prioritization of environmental health indicators.

METHODS

Models were constructed using two categories of data from publicly available datasets: public health outcome data and predictive factors. In the U.S., health departments from each

state collect vital statistics, which include birth and mortality data. The Wisconsin Department of Health Services (WDHS) maintains a database of vital health statistics aggregated by counties, regions, and sometimes city or ZIP code levels. This data is meant to help policy makers and researchers track human health and investigate causal relationships between human health and independent factors. This study investigated the relevance of these regularly collected data to risk assessments of environmental stress.

Public health data were sourced from the Wisconsin Interactive Statistics on Health Database (WISH) (WDHS 2012a), which allows queries of birth outcomes, causes of death, and cancer incidences (Table 15A). Public health data for Dobrogea were provided by the Romanian National Institute of Statistics (Table 16). Distributions of These health data were evaluated using JMP 9 (SAS institute, 2010) and transformed to adjust for heteroscedasticity (log10 for quantities and arc-sin for proportions) prior to analysis. Unsupervised learning (SOMs) was applied to two sets of vital statistic data: birth outcomes and death causes. The K-means self-organizing map clustering feature of JMP 9 (SAS Institute, 2010) was used to categorize data into clusters that described groups of counties or civil divisions with similar patterns of cause-of-death or of birth outcomes. Both cluster groups used data aggregated over 2002-2006 for Wisconsin and 2007-2010 for Dobrogea.

Supervised learning, in which output variables are defined during construction of the model, was applied to predictive variables and county clusters in the form of an artificial neural network prediction model constructed in JMP 9 (SAS institute, 2010). Predictive models were created for each set of clusters using behavioural/demographic, and landscape characteristics. The K-fold holdback validation method was used to provide model verification. Predictive factors aggregated by Wisconsin county were drawn from: US factfinder2: 2010 American Community Survey (ACS) 5-year estimates (Census 2012) and the Center for Disease Control

(CDC) Behavioral Risk Factor Survey (BRFS) 2002-2006 aggregates queried from WISH (WDHS 2012a) (Table 15B). Predictive factors for Dobrogea were aggregated by river basin landscapes within which the civil divisions were located (Shaker et al. 2010). Generalized land cover was calculated using data for 2000 (percent agriculture, forest, wetland, water and artificial/urban) for each sub-basin at 30 × 30 m resolution using ArcGIS (ESRI 2010). Nitrate and fecal coliform levels from well water were provided either by Constanta County Department of Public Health or interpolated using landscape predictive modelling (Shaker et al. 2010).

RESULTS

Wisconsin Counties

Three unique SOM clusters for counties were created describing cause-of-death patterns similar in multivariate space (Figure 8A). Among these clusters is a dominant pattern of lower medium and higher rates of death causes counties, particularly cancer related deaths (Figure 9). Death clusters 1 and 2 demonstrated patterns of higher death and cancer rates while cluster 2 was characterized by higher rates of Alzheimer's, Parkinson's, and "Other" related deaths (Figure 8A). Cause-of-death SOM clusters showed a strong spatial pattern in Wisconsin (Figure 8B) with cluster 1 predominantly located in the northern counties, cluster 2 in the south, and cluster 3 interspersed in association with major urban areas (Figure 8B). These patterns imply differing distribution for common cause of death by geography (easily seen in Figure 8B).

SOM clustering of birth-outcome data also produced three groupings of counties (Figure 10A). Birth outcome clusters showed unique patterns of higher negative birth outcomes, such as low birth-weight, in cluster 1 (Figure 11) compared with cluster 2 and cluster 3. Cluster 3 was also associated with counties having male-biased gender ratios. The spatial patterning of Birth outcome SOM clusters indicated that counties with the poorest outcomes are spread throughout the state (Figure 10B).

Table 17 displays the training and validation generalized R^2 as an indication of model performance. The Generalized R^2 uses the 2/n root of the likelihood. It is scaled to have a maximum of one (perfect model). The models with strongest predictive performance between training and validation were the models that employed behavioral risk factors as predictors. Population and land cover showed stronger predictive performance for cause of death patterns than birth outcomes. In general, cause of death seemed more sensitive to the tested variables than birth outcomes. Mother characteristics resulted in the strongest predictive value for birth outcomes, which was comparable to the cause of death analysis (Table 17). In both cases, Toxic Release Inventory data was a poor predictor of human health patterns.

The prediction profilers in Figure 12A demonstrate sensitive relationships between landscape characteristics and cause of death within a county. Prediction profiles of cause-of-death clusters indicate that clusters are particularly sensitive to median age and income (Figure 13A). For cause of death data, Cluster 1 membership is more likely with increasing median age, Cluster 3 membership with decreasing age. Median age for counties in Cluster 2 is between the other two clusters (Figure 13A). Furthermore, counties are more likely classified as Death Cluster 2 with higher percentages of people who are overweight, who have lower household incomes, and higher percentages of percentages of heavy drinkers. Conversely, counties demonstrated higher probability of Cluster 3 membership if they had higher incomes and more people who reported recent exercise (Figure 13A). The non-linear relationships described in this model imply that behavior and factors such as age influence variations in death causes within these populations. Emissions were weaker predictors of county death patterns than behavior and demographics, but some probabilistic relationships described in the model are suggestive (Figure 14A).

Land cover was not a good predictor of birth outcome patterns in Wisconsin counties (Table 17, Figure 12B). The model using landscape variables to predict county birth outcomes (Figure 12B) yielded the least informative relationships of the four models. Figure 13B illustrates the probabilistic relationships between mother characteristics and birth outcomes and was the most predictive model for birth outcomes. These predictive profilers identify increased smoking as a risk factor for [negative] birth outcome in cluster 1 and lower smoking leading to a higher probability of cluster 2 and 3 memberships. Though models using pollution data at the county level (emissions and toxic release inventory) showed weaker predictive power than mother characteristics, increased probability of counties with poorer air quality existing in cluster 1 support the notion that certain emissions could influence risk to infant health (Figure 14B).

Dobrogea Romania Civil Divisions

Four unique SOM clusters of Romanian civil divisions from Dobrogea region were identified using vital statistics and census data (Figure 16A). Cluster 1 includes civil divisions with highest rates of infant mortality and lowest birth rates. By comparison, Cluster 3 exhibits high infant mortality, but possesses higher population density and moderately high birth rates. Cluster 2 consists of civil division with older residents and higher overall death rates. Cluster 4 includes communities with a younger population and high birth rates. The geographic distributions for clusters reveal higher numbers of Clusters 1 and 2 in the northern region with an increased number belonging to Clusters 3 and 4 in the southern region (Figure 16B).

Cluster memberships were predicted using supervised learning with land cover, together with nitrate and fecal coliform levels from drinking wells were aggregated by river basins (Shaker et al 2010). Geographic variation in nitrate and faecal coliform levels is shown in Figure 16A. Well water characteristics together with land cover yielded the second strongest predictive model of cluster membership (Table 17). The risk profiles identified risk sensitive

relationships for Cluster 1 with high nitrate concentrations (Figure 16B). Cluster 3 was best predicted by high fecal coliform levels and non-urban, un-forested, low agriculture land cover, dominated by more grasslands and water (Figure 16B). Civil divisions were most likely to be in Cluster 4 when associated with moderate nitrates, low fecal coliforms in combination with mixed urban-agriculture-forested land covers.

DISCUSSION

Predictive Indicator Patterns

The two-step process of SOM-clustering health outcomes combined and supervised learning to develop risk profiles was successful in identifying key factors related to public health both in Wisconsin and Romania. In Wisconsin factors related to alcohol consumption, body weight, age and income exhibited strong risk relationship with patterns in county-level cause-of-death and birth outcomes (Figures 13A). In Dobrogea Romania, a transitional region in a developing country, drinking water quality (nitrates and faecal coliform) was shown to be sensitive risk factor in predicting infant mortality. In both cases, the spatial distributions of health outcomes revealed strong associations with demographic patterns (age distributions) and urban growth. These risk relationships are not surprising in their own right and corroborate what one might expect to find when comparing diverse communities that vary socio-economically. However, the intent of this study was to demonstrate the power of the two-step approach to identify risk profiles even when dealing with data collected across diverse regions and cultures.

It's quite reasonable to assume environmental risk factors interact with other stressors to produce complex risk relationships with human health, and one should not assume that stress-response relationships of pollutants and health can be easily patterned with linear models. Traditional methods for evaluating human health risk from environmental hazards (calculating

no observed adverse effect levels and risk ratios) tend to avoid notions of complex interactions and nonlinear systems. From hazard identification to dose-response evaluation, the standard guidelines (NRC, 1983) leave little room for an understanding of risk propagation in a dynamic system. The strength of the standard perspective is that these methods translate well for legislative and medical goal setting or decision-making. However, humans inhabit a complex landscape with multiple stressors and exposure pathways. Therefore, robust quantitative methods are needed to aid indicator prioritization and hazard identification under great uncertainty.

Predictive analytics employing both supervised and unsupervised learning techniques could meet the above goals because: (1) they easily model nonlinear relationships or interactions among variables, (2) performance is usually better than general linear models, and (3) they easily model complex data patterns. Dose-response and causal assessments may always require a more focused (or reductionist) approach. Indeed, risk ratios and reference doses are easily interpretable for health care or policy purposes. However, a predictive analytic approach may prove incredibly useful for indicator prioritization and hazard/problem identification. For example: Figure 13Aand 13B suggest that behavior, age, and income play an important role in health outcomes at a population level. This reinforces the importance of recording and monitoring these statistics to guide public health policy. It also corroborates established understandings regarding the importance of social determinants of health (Robert and Booske 2011; Vila *et al.* 2007; WDHS 2012b) and the fact that Wisconsin is among the top three States with the highest health disparities (Webb et al. 2011).

The model described in Figure 13B works because the state health department diligently collects and reports data on mother characteristics as each infant is born. If data on chemical contaminant body-burdens of mothers were collected and kept in large standardized datasets,

the same techniques could be used to investigate risks to infant health from pollution. Such a process could help direct efforts to collect and maintain databases with appropriate biomonitoring and health outcome data. With predictive models, population-level morbidity data could be sufficient to suggest data collection priorities.

Some examples of current efforts to build relevant, standardized databases include: the Wisconsin Pediatric Cardiac Registry (WPCR, 2011), National Children's Study (NCS, 2011), Exposure Profiling (Malecki et al., 2006), and the State Environmental Public Health Indicators Collaborative (SEHIC) (CTSE, 2012). These efforts underscore the need for robust, quantitative methods to aid decision-making, prioritization, and hazard identification. In order to make the best use of health databases it will be necessary to employ techniques sophisticated enough to model complex interactions and associations. Ultimately, unsupervised and supervised learning techniques present powerful methods that can pattern data in almost any way desired. For example: back propagation networks with biases, a sigmoid layer, and linear output layer are capable of approximating any function, linear or nonlinear, with a finite number of discontinuities (Novotny et al., 2009). Therefore, sensible interpretation of risk propagation frameworks will be paramount.

As reviewed in Chapter 1, the fields of environmental toxicology and risk assessment face new and growing challenges due to issues such as chemicals of emerging concern and uncertainty over ambiguous or limited hazard and exposure information. The methods demonstrated here can serve to facilitate screening of multiple and interacting hazards to human populations and aid in the prioritization of health indicators. Larger, standardized databases that are targeted towards pollution and health would allow for a predictive analysis thereby producing a proactive approach to hazard identification. Finally, predictive analyses of

diverse, standardized datasets would also aid in the tracking of progress towards a cleaner and healthier environment.

Table 15: Statistical data used for (A) SOM Clustering of public health indicators, and (B) Directed-learning Neural-network prediction of clusters. Wisconsin data were queried from Wisconsin Interactive Statistics on Health database by county for years 2002-2006. Data for civil divisions in Dobrogea Romania were provided by the Romanian National Institute of Statistics. Unless otherwise noted, data were calculated as percent and arc-sine transformed prior to analysis. Death Rates were calculated per 100,000 population and Infant Mortality rate is per 1,000 live births. Sex ratio is the number of male birth divided by total births.

A. Population-level Health Outcomes

Causes of Death and Cancer (WI Counties N=72)	**Birth Indicators (WI Counties N=72)**	**Vital Statistics (RO Civil Divisions N=126)**
Cancer Incidence	Infant Mortality	Infant Mortality
Cancer	Premature Births	Birth Rate
Alzheimer's and Parkinson's	Low Birth weight	Death Rate (non-infant)
Diabetes	Sex Ratio Male	Median Age (years)
Heart Disease	Fertility	Population (per km^2)
Lower Respiratory Disease		
Other Death		

B. Predictor Risk Variables (WI)

Behavioral Risk Factor Survey	**Maternal Factors**	**Toxic Releases (g/km^2)**	**Census Data**	**Emissions (g/km^2) (2002)**	**Land Cover**
Smokers	35 or older	OSHA Carcinogens	Median Age (years)	Carbon Monoxide	Urban
Obese	Smoked	Hazard Air Pollutants	Population (per km^2)	Mono-Nitrogen Oxides	Agriculture
Overweight	No College	Persistent, Bioaccumulative, Toxics		Volatile Organics	Forested
Heavy Drinkers	Kessner Index: poor care	Metals		Sulfur Dioxide	Grassland
Exercised in the last 30 days	Caucasian			Particulate Matter <2.5 microns	Urban
	Birth Interval <2yr			Particulate Matter <10 microns	Water, Wetland
				Ammonia	Barren

Table 16: Statistical data used for Directed-learning Neural-network prediction of clusters (Romania). Data for civil divisions in Dobrogea Romania were provided by the Romanian National Institute of Statistics.

Predictor Risk Variables (RO)		
Census Data	**Drinking Water Quality**	**Land Cover Data**
Median Age (years)	Nitrate (mg/L NO3)	Urban
Population (per km^2)	Fecal Coliform (#/100ml)	Agriculture
		Forested
		Grassland
		Urban,
		Water, Wetland

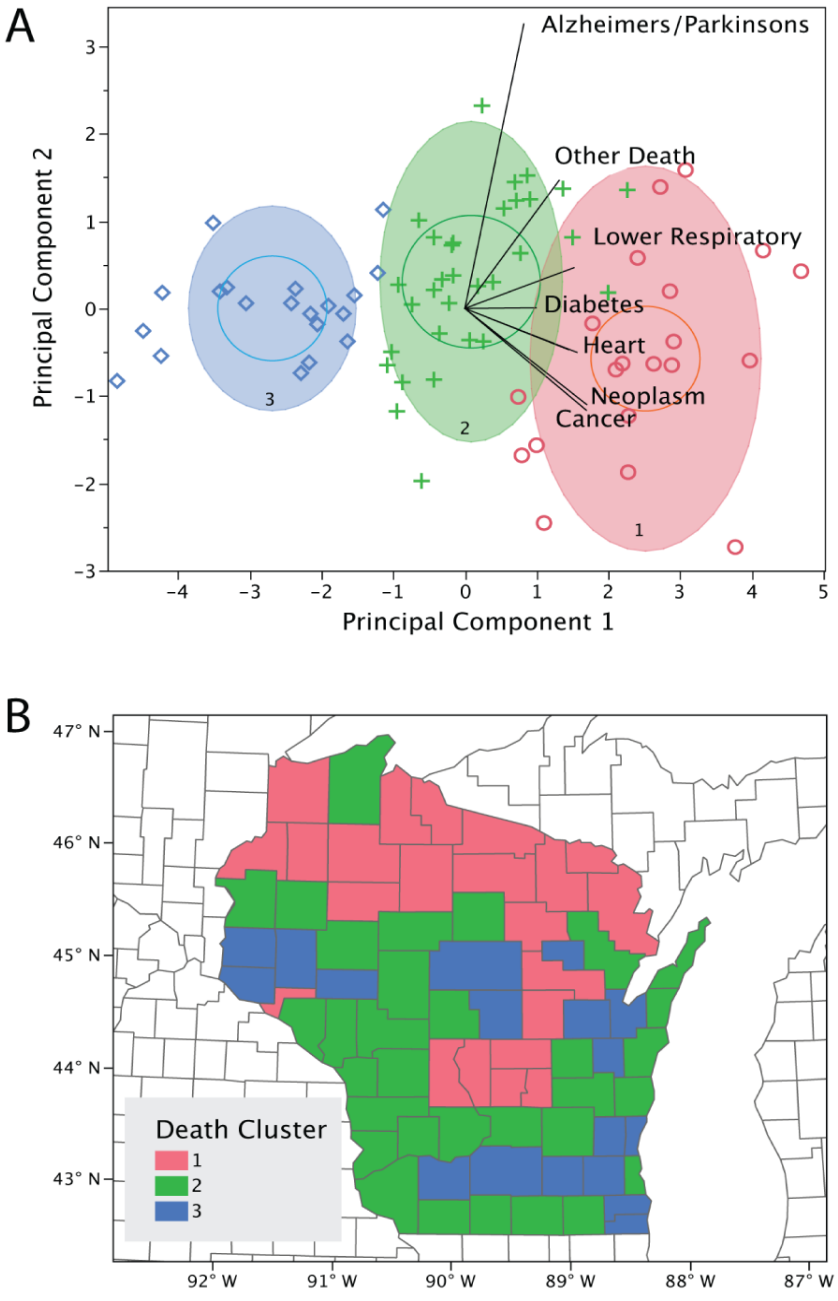


Figure 8: Statistical Clustering and Spatial mapping of County-level Cause-of-Death data for Wisconsin, USA. Figures show Biplots of SOM clusters with rays indicating relationship relative to standard Principal Component eigenvectors, and maps SOM Clusters color-coded for Wisconsin Counties. Cluster 1 = red circles, Cluster 2 = green crosses, Cluster 3 = blue diamonds. **(A)** Cause-of-Death SOM Biplot; **(B)** Cause-of-Death SOM Map.

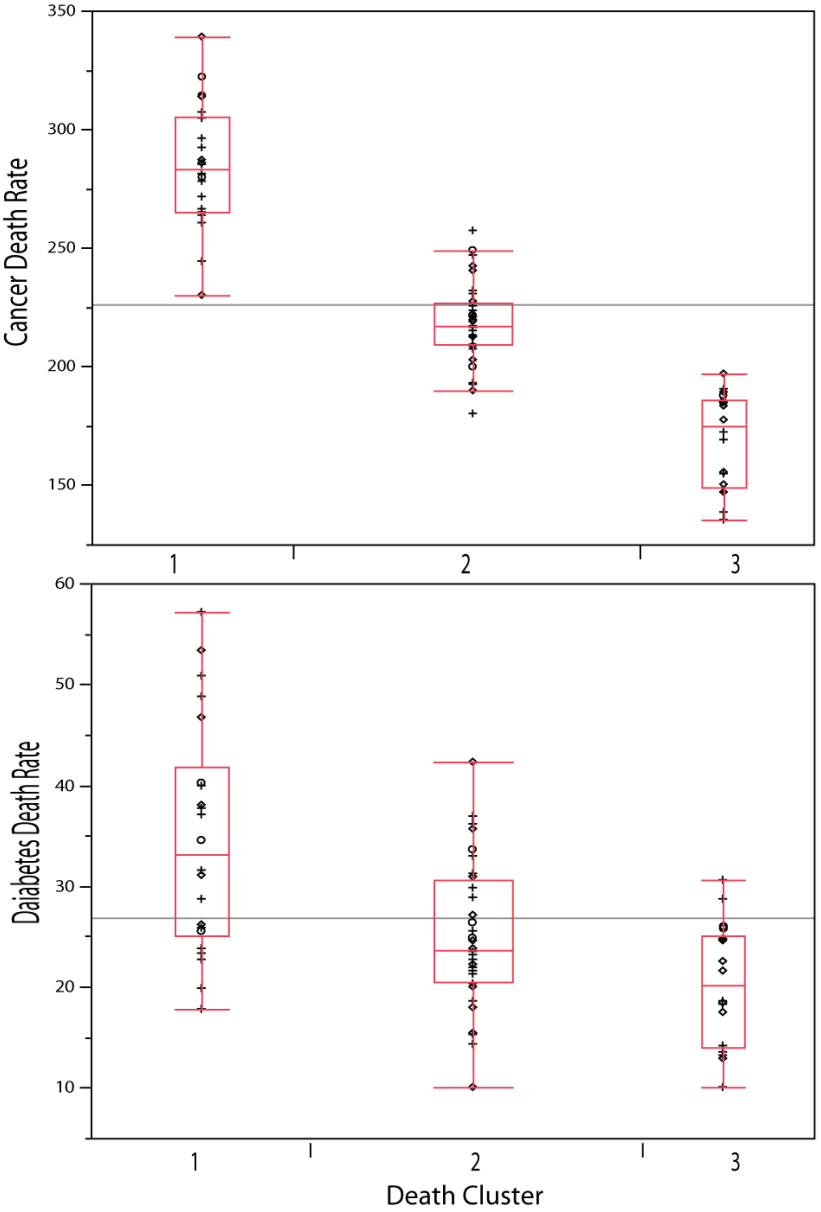


Figure 9: Box plot displaying selected death rates (Cancer and Diabetes) across SOM clusters which were created using birth vital records from Wisconsin counties.

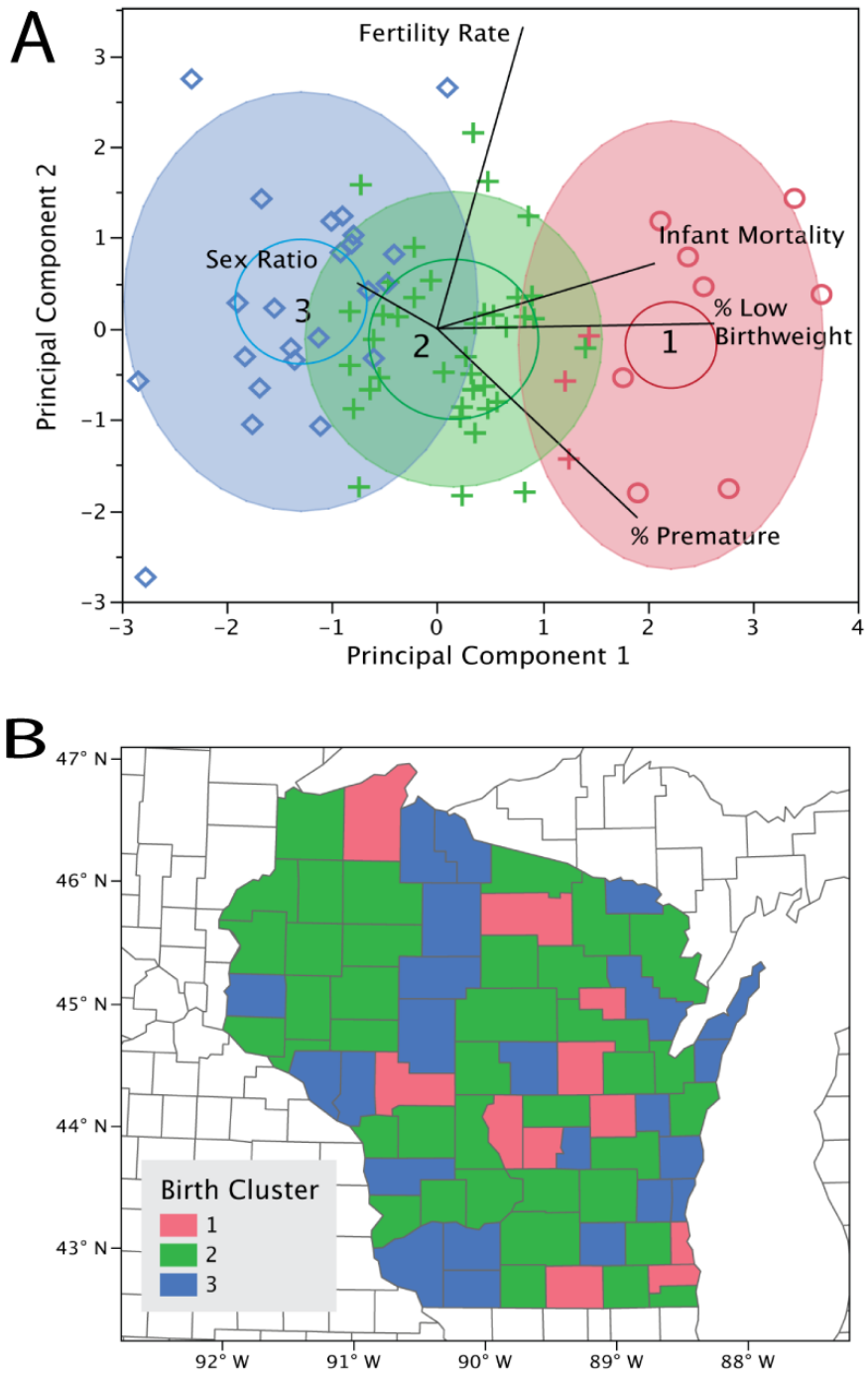


Figure 10: Statistical Clustering and Spatial mapping of Birth Outcomes data for Wisconsin, USA. Figures show Biplots of SOM clusters with rays indicating relationship relative to standard Principal Component eigenvectors, and maps SOM Clusters color-coded for Wisconsin Counties. Cluster 1 = red circles, Cluster 2 = green crosses, Cluster 3 = blue diamonds. **(A)** Birth Outcomes SOM Biplot; **(B)** Birth Outcomes SOM Map.

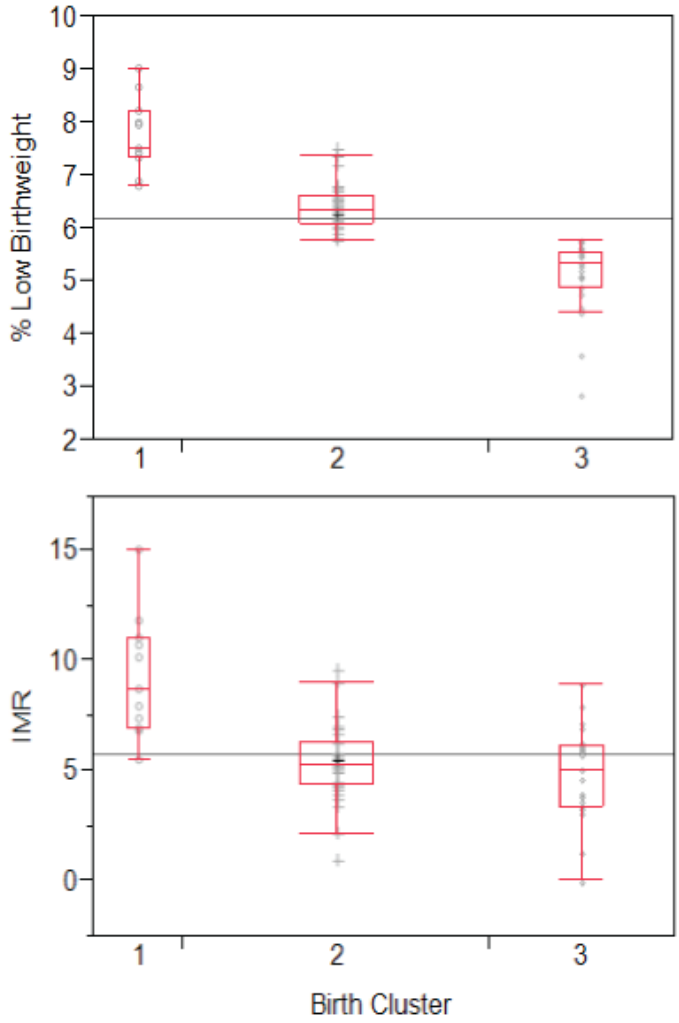
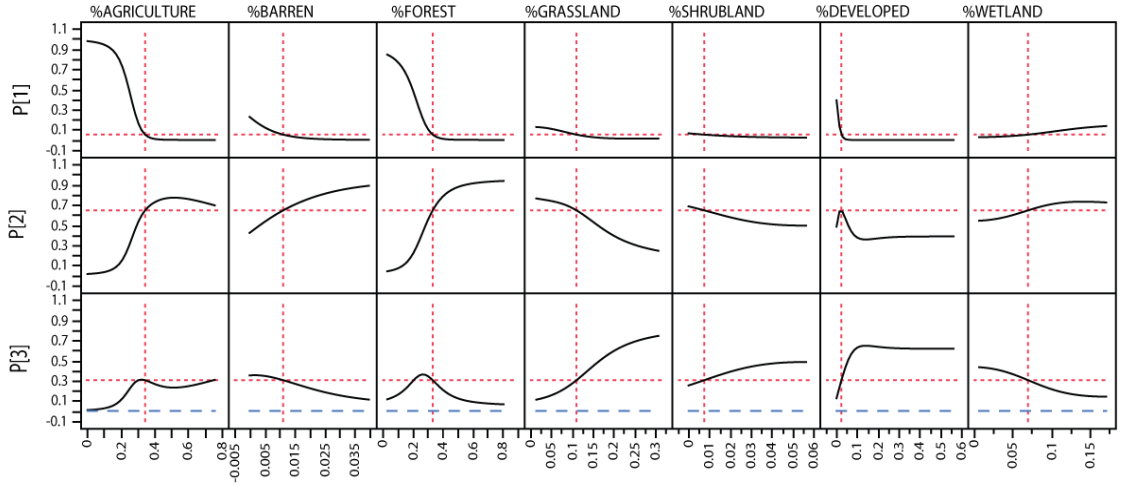


Figure 11: Box plot displaying selected birth outcomes (percent low birth weight and infant mortality rate) across SOM clusters which were created using birth vital records from Wisconsin counties.

Table 17: Predictive models for two types of health-outcomes and two types of predictive factors. Training and Validation R^2 are reported which use the 2/n root of the log-likelihood. The R^2 is scaled to have a maximum of 1 for perfect model and 0 for a model no better than a constant model.

Model Inputs	**Model Output**	**Training Generalized R^2**	**Validation Generalized R^2**
Land Cover	Cause of Death Clusters (WI)	0.748	0.603
BRFS and Demographics	Cause of Death Clusters (WI)	0.854	0.901
Emissions	Cause of Death Clusters (WI)	0.533	0.736
Toxic Release Inventory	Cause of Death Clusters (WI)	0.409	-0.079
Land Cover	Birth Outcome Clusters (WI)	0.245	-0.221
Mother Characteristics	Birth Outcome Clusters (WI)	0.707	0.775
Emissions	Birth Outcome Clusters (WI)	0.355	0.443
Toxic Release Inventory	Birth Outcome Clusters (WI)	0.261	0.423
Drinking Water Quality and Watershed Land Cover	Vital Statistics (RO)	0.823	0.762

A. Landcover Prediction Profiles: Wisconsin Counties Clustered by Cause of Death



B. Landcover Prediction Profiles: Wisconsin Counties Clustered by Infant Vital Statistics

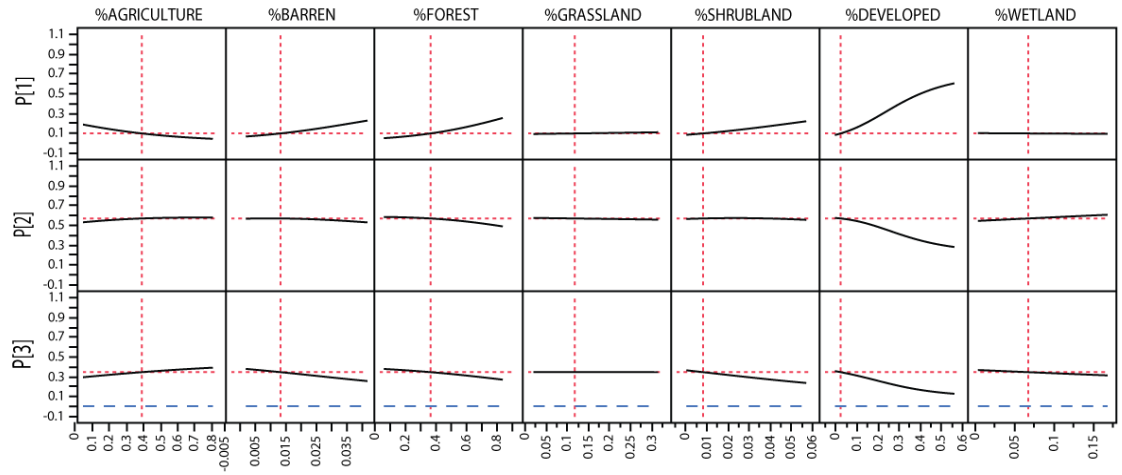


Figure 12: Categorical profiler of cluster prediction using seven land cover variables for Cause of Death clusters (A) and Birth Vital Statistic clusters (B). These are two separate models. X axis indicates percent of county land covered. Y axis indicates fitted probability of the response category.

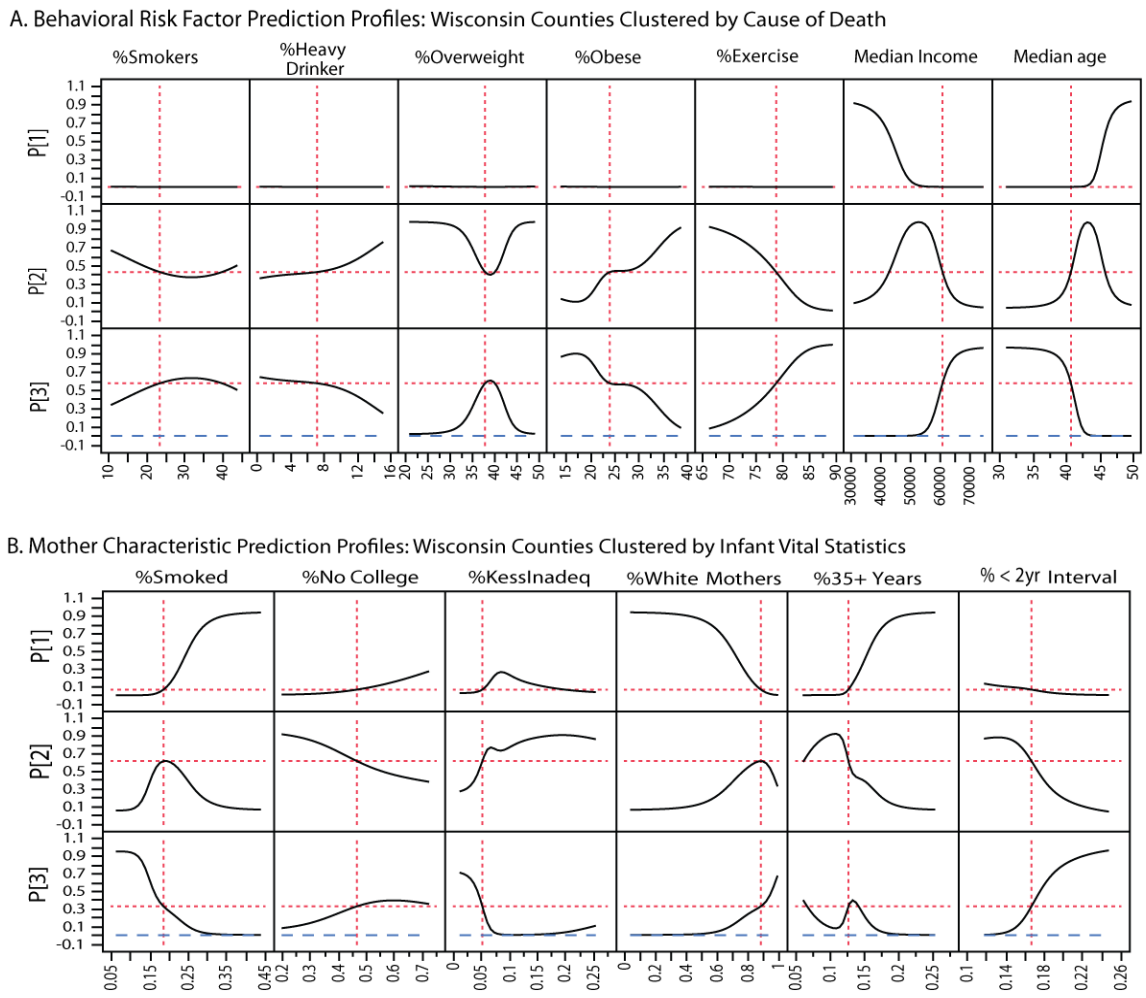


Figure 13: Categorical profiler of cluster prediction using six mother characteristics factors (A) and behavioral risk factors (B). These are two separate models. X axis indicates percent smokers, heavy drinkers, overweight obese, exercised in the past 30 days, median income and age (A); percent mothers within county who smoked, had inadequate or no prenatal care (Kessner index), where 35 or older, who's birth interval was within 2 years, are white, and did not attend college (B). Y axis indicates fitted probability of the response category.

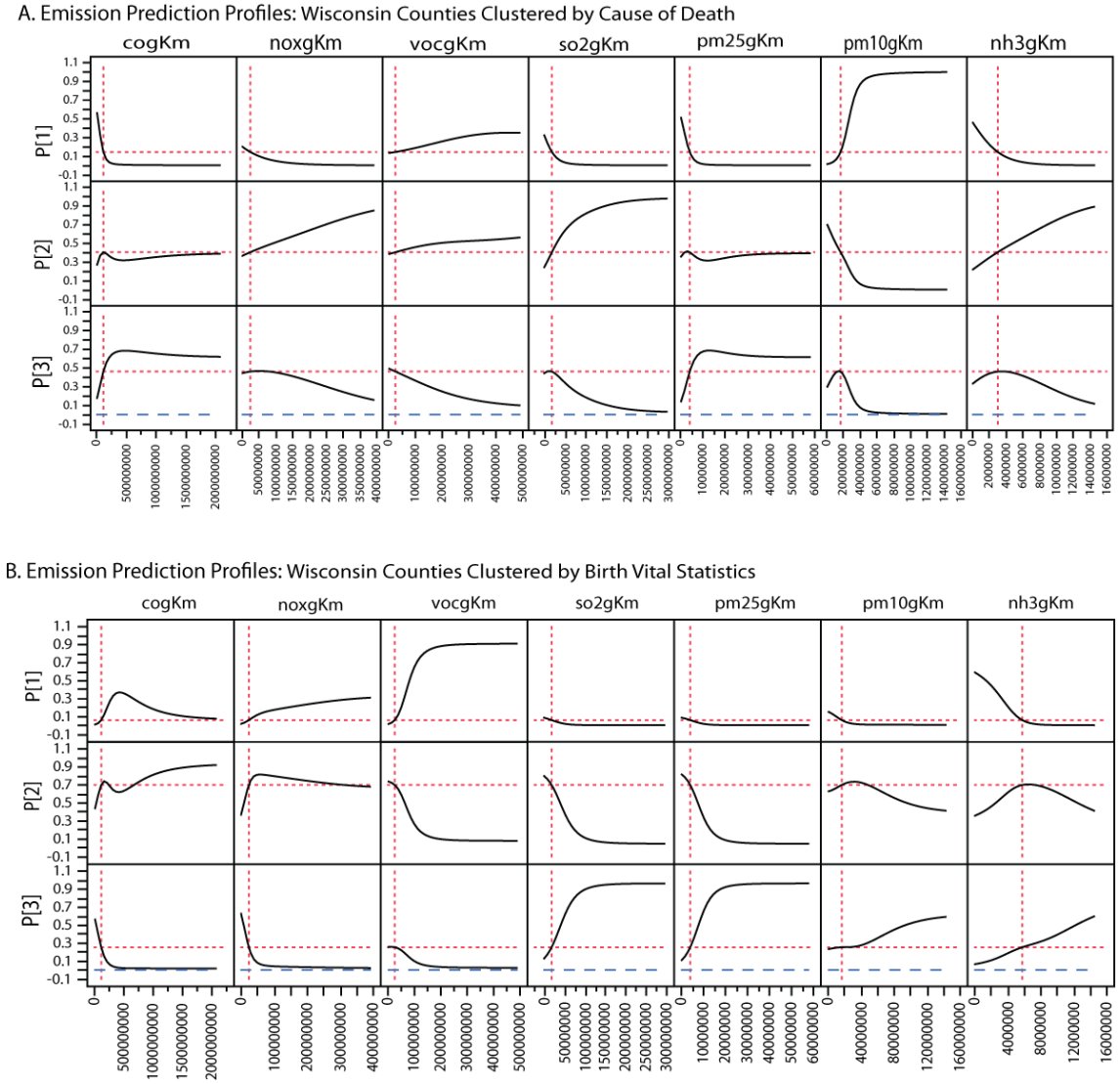


Figure 14: Categorical profiler of cluster prediction using seven air quality variables for Cause of Death clusters (A) and Birth Vital Statistic clusters (B). These are two separate models. X axis indicates grams per kilometer of carbon monoxide, mono-nitrogen oxides, volatile organics, sulfur dioxide, particulate matter <25 microns, particulate matter <10 microns, and ammonia. Y axis indicates fitted probability of the response category.

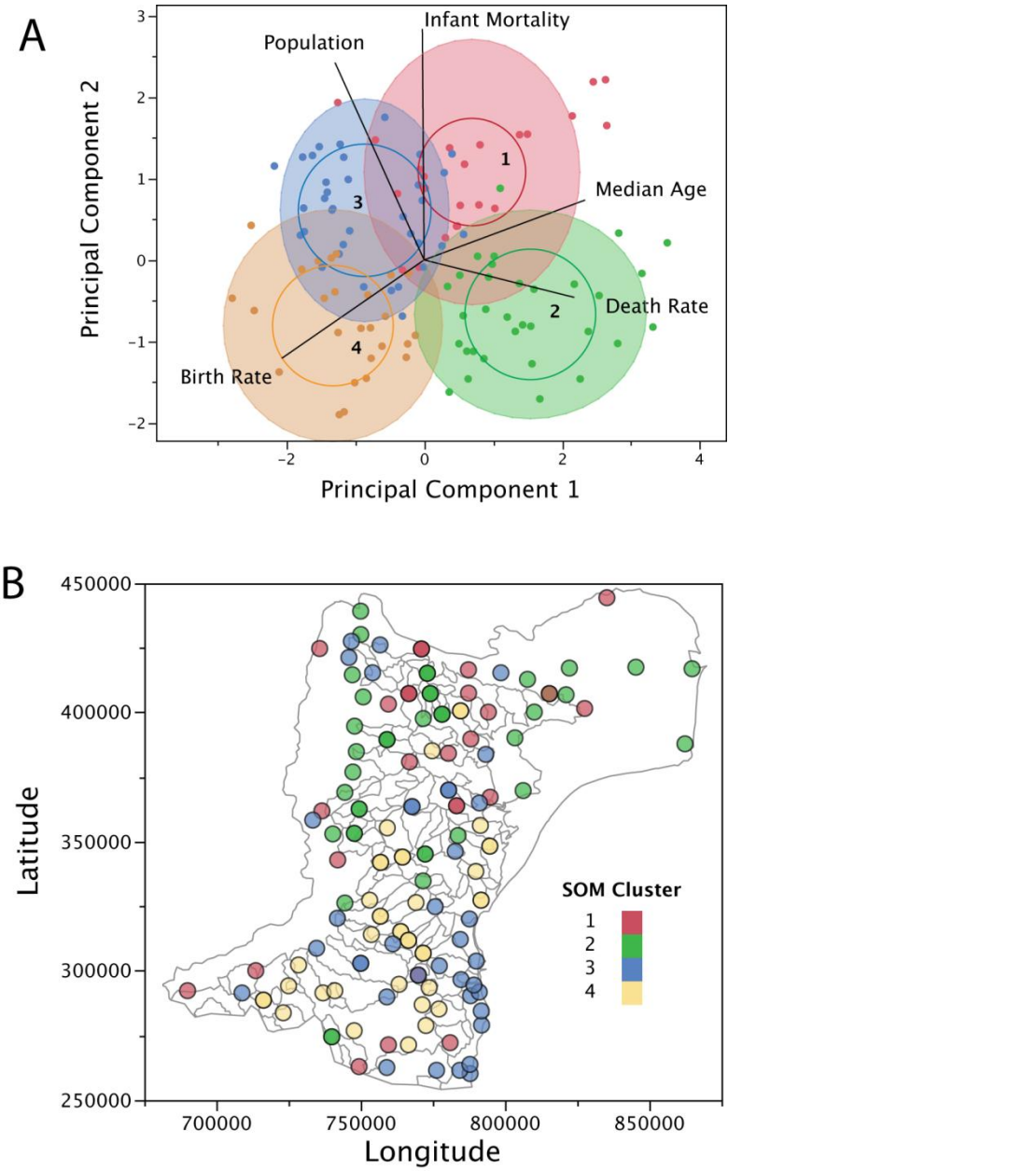
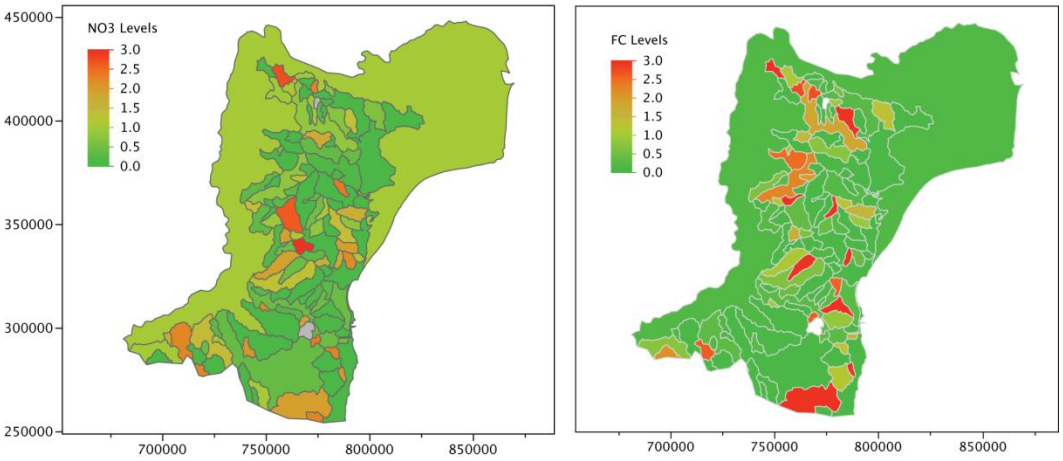


Figure 15: Statistical Clustering and Spatial mapping of Civil Division-level Vital Statistics data for the Dobrogea region of Romania. Figures show Biplots of SOM clusters with rays indicating relationship relative to standard Principal Component eigenvectors, and maps SOM Clusters color-coded for each Civil Division. Cluster 1 = red, Cluster 2 = green, Cluster 3, Cluster 4 = yellow. **(A)** Vital Statistic SOM Biplot; **(B)** Vital Statistic SOM Map showing river basin shapes.

A. Nitrate and Fecal Coliform Levels for Romanian Coastal Watersheds



B. Prediction Profiles: Romanian Vital Health Statistics Clusters

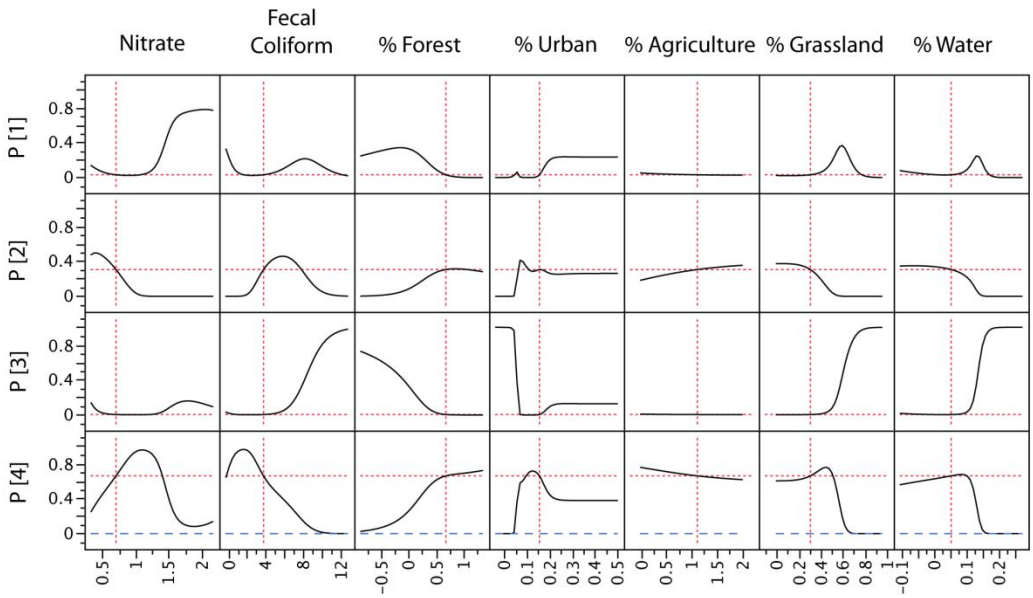


Figure 16: (A) Maps showing log10 Nitrate and Faecal Coliform levels for river basins in Dobrogea Romania calculated from Shaker et.al (2010). **(B)** Risk Prediction profiles for SOM cluster identification using directed-learning neural network models of two water quality and five land cover parameters. X-axis indicates the level of risk factors for a Civil Division. Y-axis indicates the probability for a Civil Division to be classified within a specific SOM Cluster (P[1], P[2], P[3] and P[4] respectively) when exhibiting a specific level of the risk factor on the X-axis.

Chapter 4: Prospectus for Using Predictive Analytics in Environmental Health

Chapter One reviewed the challenges of toxicology and risk assessment from the perspective of chemicals of emerging concern. The CECs present a pertinent example of uncertainty associated with complex systems in which the well-being of human and ecosystem health requires action. The Great Lakes ecosystem, including the human inhabitants, can be considered a dynamic and adaptive system. Uncertainty regarding the factors that influence environmental and public health is increased through a variety of issues: complex interactions, limited knowledge, logistical, moral and political constraints to research. To some extent, many of these restrictions are absolute. This permanently increases uncertainty regarding human health and the environment. On the other hand, perception of uncertainty may depend on one's point-of-view and accepted philosophies.

Knowledge built towards evaluation of toxicological causality results in conclusions containing a caveat of uncertainty. In the context of quantifying health risks within dynamic systems, uncertainty may be considered as a reductive quantification of phenomena which actually describe plasticity, adaptability, or even chaos. The techniques I described in Chapters Two and Three are examples of operational tools that can inform sustainable and protective practices through integrative Risk Assessment. Much of the research cited in Chapter One underscore a need for the application of dynamic approaches to accumulating knowledge and working towards consensus. Knowledge and consensus do not necessarily adhere to the bounds of certainty. System-oriented analysis may provide 'answers' (perhaps more appropriately termed: guidance) that encompass multiple outcomes, qualitative directives, and context-dependent ideas.

As previously discussed, current trends in policy-oriented research indicate a strong demand for integrative indicators that quantify conditions relevant to human health and the

environment. For example, the recent amendment (September 2012) to the Agreement between the United States of America and Canada on Great Lakes Water Quality calls for:

> "evaluating the effects of chemicals of mutual concern, and combinations thereof, on human health and the ecosystem, including the development and use of reproductive, physiological and biochemical measures in wildlife, fish and humans as health effect indicators;" (emphasis added)

The National Health and Environmental Effects Research Laboratory in the Environmental Public Health Division of EPA, is engaged in research aimed at developing a measure that estimates overall environmental quality at the county level for the United States (EPA 2012). The project (EPA 2012) expresses a need for efforts to learn more about how various environmental factors contribute to health disparities in low-income and minority populations. EPA is therefore conducting an ongoing project to create an Environmental Quality Index which resembles the county-level work conducted in Chapter Three of this dissertation. ZSCA (discussed in Chapter 2) can serve as a holistic indicator of environmental health and predictive analytics (discussed in Chapter 3) can be applied to this and other indicators to investigate the complex relationships between society, health, and environment.

Systemic and Predictive Analytics to Generate Knowledge

Development of systems-oriented analytical tools can allow for effective use of publicly collected data. The neural network analysis in Chapter Three demonstrates interesting public health patterns in the State of Wisconsin using vital statistic data available to the public. The models described increasing probability of cluster membership in association with risk factors thought to relate to public health measures. Many of the measures used for vital statistics have been investigated and associated with environmental stress (Table 18).

Using death and birth records to create public health patterns may provide useful human indicators for study, which would represent dynamic system outcomes. I was able to find non-linear relationships between air quality and death rates; however the results from Chapter Three suggest that vital statistics may be more useful for tracking social determinants of health than environmental determinants. Nevertheless, a sensible interpretation of this is that both socio-economic status and factors such as air quality interact to influence public health patterns. As seen on Figures 17, 18, and 19 negative birth outcomes are higher in counties with higher proportions of minorities (Racine, Milwaukee, Menomonee) or sparsely populated counties (Iron, Bayfield, Forest). It would be interesting to observe patterns of morbidity data on birth defects like CHD, cancer, auto-immune diseases, or endocrine-related diseases compared with integrative measures of environmental conditions in these areas.

EDA and the Screening of the Human Environment

As previously discussed, uncertainty and complexity lead human health investigations towards assessment of risk and not necessarily cause. The study of risk allows for the characterization of 'stressors'. Stressors are agents that increase the risk of a negative health outcome (EPA 2008; NRC 1983). These agents are usually not considered causal, but are somehow related to the cause in a meaningful way.

Studying human response to complex environmental stress requires the quantification of environmental conditions. In the literature, characterization of environmental condition can take many forms. Many of the recommendations from the toxicological profiles of PBDEs, for example, are derived from studies using animal models. Limited information is available on thyroid effects in PBDE-exposed humans (ATSDR 2004). Recommendations on mercury, however, are often based on human cohort data in which exposure was characterized via sampling of Hg in hair (Davidson 1998). Furthermore, there exists epidemic-level evidence of

mercury exposure and health effects from Japan (Minamata disease); in which seafood contaminated by industrial Hg pollution created an entire generation of congenital deformities as well as acute toxicity in cats (Nakano 2010).

Exposure assessment, a key element of HHRA protocols, remains an elusive factor within an EDA screening framework (Salmon 2010). If effect-screening uses aggregate data to link humans to regional variations of environmental stress, identification of exposure routes may be impossible. Studies using aggregated data are often deemphasized due to a limited ability to infer causality for individuals; but this assumes conclusions for the individual are necessary for useful policy and/or assessment. This need not be the case. Researchers are recognizing the need to consider effects at a population level (Dunn and Alexeeff 2010; Faust 2010). Restructured risk-assessment frameworks (e.g. Figure 20) can produce a probability-based linkage between individual-level assessments and population-level assessments. Exposure vectors for populations are certainly related to the individual exposure routes, but both are distinct. Hypothetically, a population-level assessment could investigate all local pollution sources as they may manifest in environmental media. Keiter *et a*l (2009) were able to use EDA to identify potential sources of toxic stress to fish populations in the Danube River for example. Monitoring efforts designed this way could prevent occurrence of tragic epidemics and aid in the study of environmental stress to human populations. Perhaps most impactful, this perspective is the most likely to produce the kinds of indicators needed by policy-makers.

I used the zebrafish sediment contact assay (ZSCA) from Chapter Two to evaluate the possibility of characterizing toxic effects within the environment that would be applicable to human populations. Most development in toxicity screening focuses on testing various methods to respond to environmental stress, but not to characterize differences across human boundaries (Hallare *et al.* 2005; Hallare *et al.* 2011; Kuch *et al.* 2010; Rocha *et al.* 2011; Strmac

et al. 2002; Yu *et al.* 2008). The version of ZSCA applied in Chapter Two was specifically designed for comparison to public health. This is a separate directive than toxicity screening for specific chemicals or hazards; though it can and should inform both processes. More specifically, this genre of toxicity screening is designed to take part in larger systemic information frameworks.

Such frameworks need to be supported by various forms of evidence, including both quantitative and qualitative forms of information. I do not assume that human political and municipal boundaries create areas or populations with any consistent relevance to human biology. However, these boundaries provide aggregations that allow human populations to be compared in a practical manner to their environments. For this reason, it may be advantageous to use multivariate techniques that quantitatively pattern biological responses. SOM clusters and principal components allow for the characterization and comparison of complex sets of variables. This allows researchers to take advantage of a large and diverse database of independent factors that may represent risk factors or response variables in the system being studied. Principal Component Analysis, for example, creates factors that explain the greatest degree of variance and therefore may represent factors which are potentially biologically relevant to the system of study.

In Chapter Two, a principal component describing cardiovascular development in zebrafish was significantly related to risk of increased CHD prevalence. That same component (PC3) was also significantly associated with PCB concentrations and organic content of sediment samples. It is possible that cardiogenesis in zebrafish responds to mixed environmental stressors in a similar manner to human heart development. There is potential to identify sensitive teratogenic stress indicators of streams in human communities. Other findings from Chapter Three suggested that currently collected human health indicators (infant mortality, low

birth weight) may share some probabilistic relationships to a generalized stress-response in zebrafish exposed to sediments in those communities. This suggests the possibility of calibrating a generalized stress-response indicator to detect environmental health disparities in human communities.

Proposed Next Steps

Public health data can be difficult to use in risk assessments. Working with large, regularly-collected, standardized datasets presents certain advantages. However, significant difficulties with this approach include: restricted access due to privacy and bureaucracy, rarity of outcomes, a lack of trust between community and researchers due to the impersonal nature of regular data collection. Novel methods for gathering health-related data may be adapted using community-based participatory research (CBPR). CBPR is used to enhance translational research and is a key process for increasing the efficacy of evidence-based practices for social change (Allen *et al.* 2010; Hacker *et al.* 2012).

Previous work using CBPR to translate research into culturally relevant fish consumption advisories has demonstrated the effectiveness of "Talking Circles" (a form of focus group) to produce culturally-sensitive information (J Dellinger *et al.* 2008; Dellinger *et al.* 2006). This format may provide useful opportunities to gather community-based health information. A health evaluation using the talking circle format will allow for a comparison of environmental health and self-reported community health within a socially distinct group: Native Americans.

The hypothetical study would focus on empowering Native American communities to self-actualize as risk assessors. Students from Tribal communities would be employed to collect sediments in local streams (similar to the methods described in Chapter Two) which would be tested for teratogenic stress. Participants from these communities would then be enrolled in Talking Circles in which measures of community health would be developed. The talking circles

methodology would provide a framework for evaluating health endpoints that are relevant to community environmental health. This information would then be quantified and compared to the environmental health assessments from the local streams.

Conclusions

When science and policy interact, one must engage in some form of probabilistic assessment. Some call Risk Assessment a ‘‘shot-gun marriage between science and the law’’ which attempts to apply scientific methods and evidence to issues of administration and legislation, yet fails to fully satisfy the needs of either the legal or scientific communities (Russell and Gruber 1987).

The ultimate goal of integrated risk assessment is to provide answers to issues requiring management, monitoring and development decisions using organized scientific frameworks; even if the answers, at least for the time being, are inexact and uncertain (Sexton 2012). Regarding the long list of chemicals of emerging concern; the current knowledge is limited. Some may interpret limited knowledge as evidence of absence. If any CECs from the list in Chapter One are *proven* to negatively impact human health, then something has gone horribly wrong.

Unfortunately, one can point to specific examples in which humans were exposed to and negatively impacted by pollution. Minamata disease (Nakano 2010), the Seveso Dioxin cloud (Laporte 1978), and Yusho/Yu-Chen disease (Aoki 2001) are a few examples of unwilling human subjects that were exposed to high levels of toxic chemicals. Though these incidences demonstrated the toxicity of Mercury, PCBs, and Dioxins; anyone would agree that the human cost of this level of certainty is unjustifiable. These realities will drive innovation in epidemiology, risk assessment, and toxicology. The techniques demonstrated in this dissertation present examples of that motivated innovation.

Future efforts in this work will require interdisciplinary collaboration and practices such as community-based participatory research. Further development of monitoring and evaluation approaches that respond to multiple forms of knowledge will best guide future practices, such as sustainable peace building. Better understanding of the system will lead to better outcomes, not just in human health, but economically, ecologically and aesthetically.

Indicator	Fisk Factors	Authors
Gender ratio (male births: female births)	Fumigants/ Pesticides, Dioxins, PCBs	(Hertz-Picciotto *et al.* 2008; Ishihara *et al.* 2007; Milham and Ossiander 2008; Mocarelli *et al.* 2000)
Infant Mortality Rates (deaths per 1,000 births)	Degraded Stream Quality, Nitrates & Microbes in Drinking Water, Agricultural Runoff, Proximity to Traffic	(Comly 1987; Gouveia 2004; Manassaram *et al.* 2006; Paul *et al.* 2008; Wallinga 2004)
Low Birth-weight/ Premature Births	Air Pollution, Traffic, Carbon monoxide etc.	(Brauer *et al.* 2006; Gouveia 2004; Gouveia and Medeiros 2003; Ritz *et al.* 2000)
Neoplasm (per 100,000 deaths)	Arsenic in Drinking Water, Urban Residence/Traffic, Parental Pesticide Exposure, Paints, Petroleum Products, Solvents, and metals.	(Coughlin *et al.* 2006; McBride 1998; McLafferty and Wang 2009; Nieder *et al.* 2009; Shim *et al.* 2009)
Pneumonitis and Pneumoconiosis (per 100,000 deaths)	Metal Dust, Animal Dander, Dust, Agriculture, Stone Dust, Air Pollution	(Baumgartner *et al.* 2000; Mapel and Coultas 1999)

Table 18: List of vital statistic indicators with suggested risk factors supported by epidemiological literature.

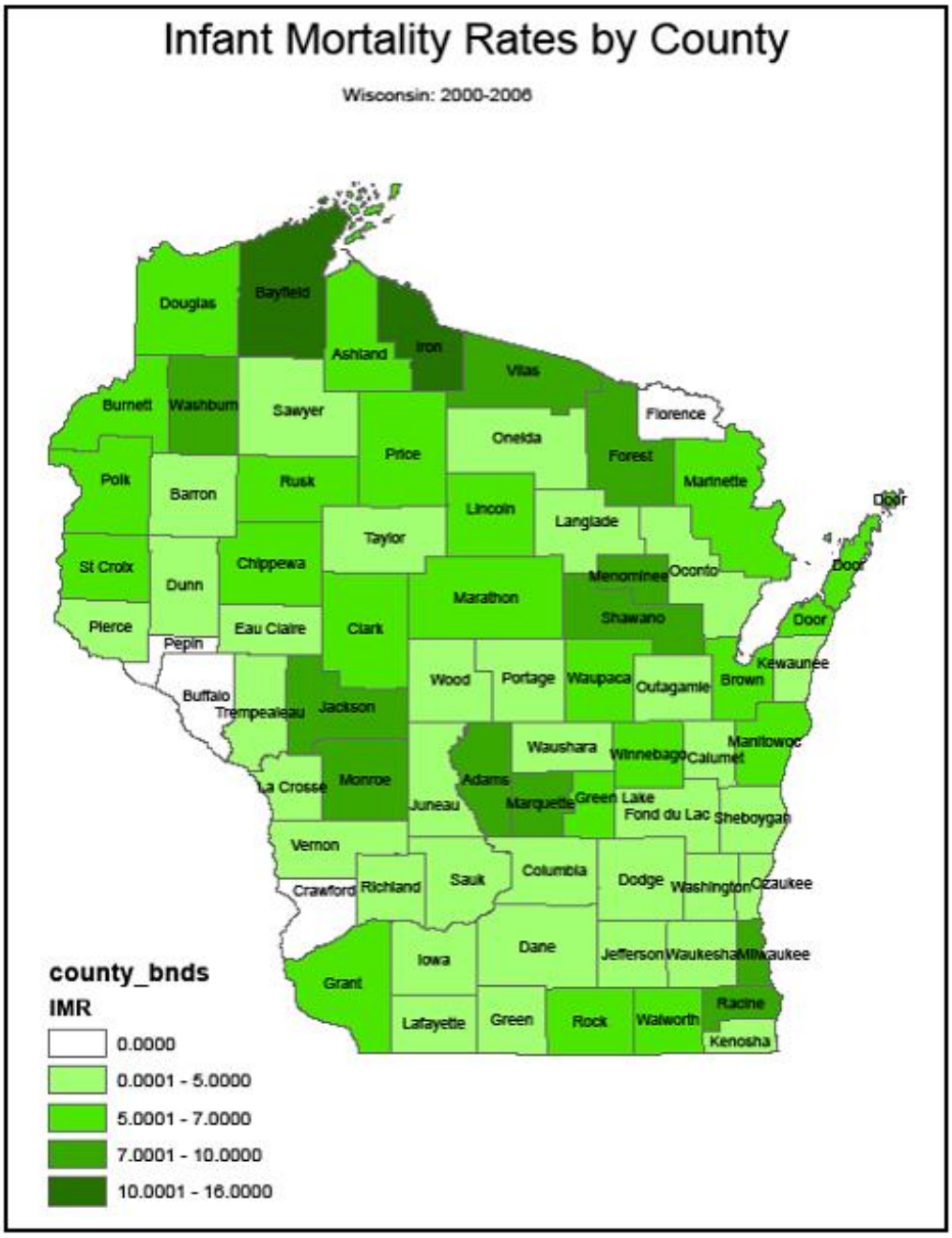


Figure 17: Infant mortality rates (IMR = deaths per 1,000 births) color coded by Wisconsin counties (years 2000-2006). Homicidal and accidental deaths were not incorporated into this database.

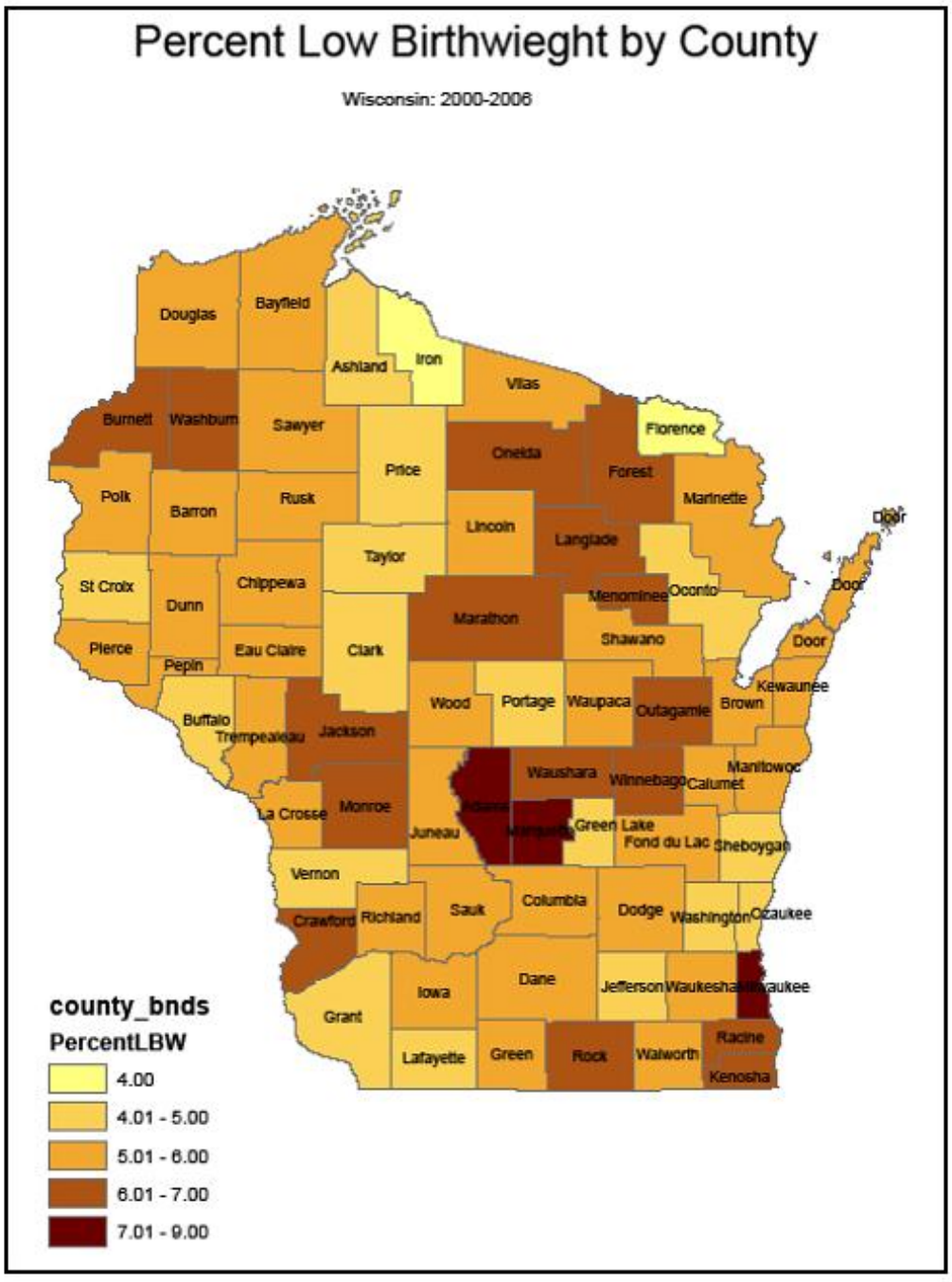


Figure 18: Percent low birthweight (less than 2,500 grams) color coded by Wisconsin counties (2000-2006

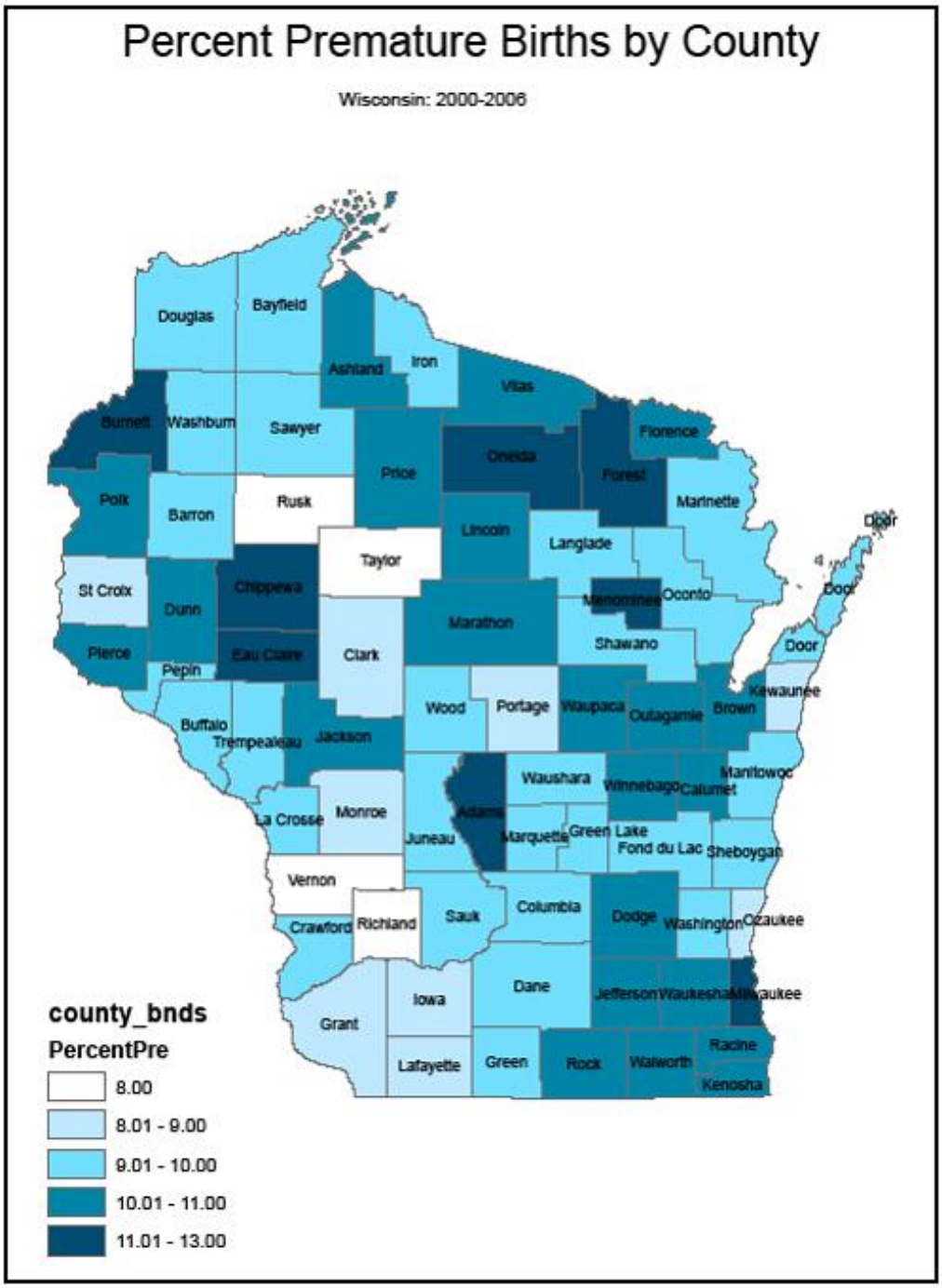


Figure 19: Percent premature births (less than 37 weeks gestation) color coded by Wisconsin counties (2000-2006).

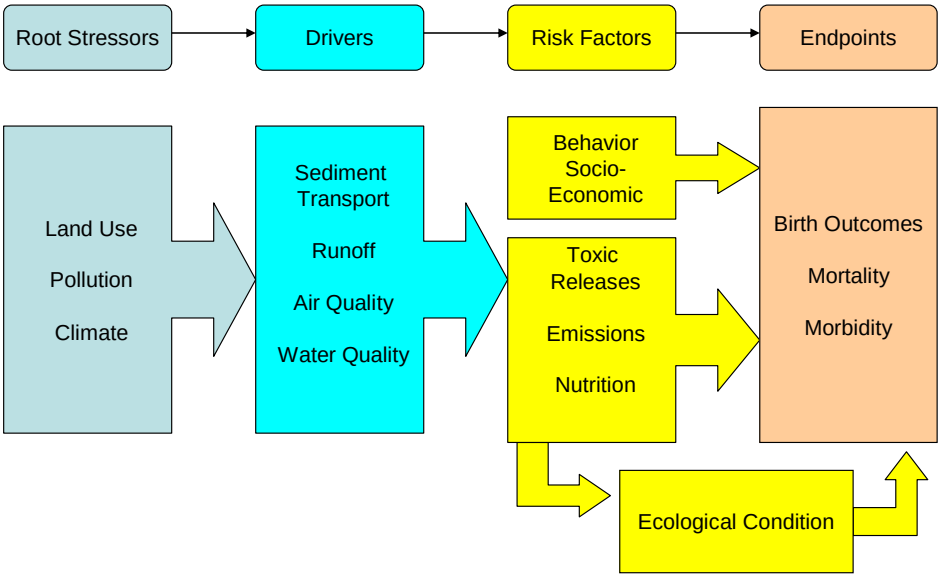


Figure 20: Proposed model showing established risk propagation from environmental stressors to ecological condition and the hypothesized relationships between environmental stress and public health.

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CURRICULUM VITAE

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Education:

2007-2012	Ph.D., Biology, University of Wisconsin-Milwaukee (December 2012)
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2000-2004	B.S., Integrated Biology, University of Illinois Urbana-Champaign

Appointments:

2012-Present	Post Doctoral Fellow, Great Lakes Inter-Tribal Council, Lac du Flambeau, WI
2007-2012	Graduate Research Assistant, Biological Sciences, University of Wisconisn-Milwaukee
2004-2007	Graduate Research Assistant, Marine and Freshwater Biomedical Sciences Lab, University of Wisconsin-Milwaukee

Other Professional and Research Experience:

2007	Human Health Risk Assessment Consultant, Symbiont Inc. West Allis, WI
2004-2007	Environmental Health Consultant, PicaTox Inc., Milwaukee WI
2001	Undergraduate Field Assistant, University of Illinois Urbana-Champaign
2001-2002	Undergraduate Lab Assistant, National Soybean Research Center, University of Illinois Urbana-Champaign

Teaching Experience:

2012	Guest Lecturer: BIOSCI 599 "Complex Social-Ecological Systems: Transdisciplinary Approaches, Resilience & Management" Undergrad/Grad, 2 Credit hours (University of Wisconsin-Milwaukee)
2011-2012	Guest Lecturer: CES 210 "Introduction to Conservation and Environmental Science," Undergrad, 3 Credit hour (University of Wisconsin-Milwaukee)
2010	TA Coordinator: BIOSCI 150 Laboratory Sections, Undergraduate, 3 Credit hours (University of Wisconsin-Milwaukee)

2008 TA/Guest Lecturer: “Epidemiology for the Health Sciences,” Undergrad, 3 Credit hours, (University of Wisconsin-Milwaukee)

2007-2010 Graduate Teaching Assistant: BIOSCI 150 Laboratory Sections, Undergraduate, 3 Credit hours (University of Wisconsin-Milwaukee)

Abstracts, Posters and Selected Presentations:

Matthew J. Dellinger, Timothy Ehlinger, Michael Carvan, and Jesse Jensen. 2012. Zebrafish Sediment Contact Assay as a Novel Tool for Integrating Human Health and Ecological Risk Assessment. Poster for May 3rd, CEHSCC All Investigators Gathering

Timothy Ehlinger, Matthew Dellinger, and Lucia Tofan. Neural Networks, Pattern Analysis and the use of Ecological Approaches to Inform Human health Risk Assessment. Presentation: June 21, 2012, BENA 2012 Istanbul Conference.

Matthew Dellinger. 2011. Using Effect-Directed Analysis in a Watershed-Based Risk Assessment. Presentation: July 12, 2011 Children’s Hospital of Wisconsin, Cardiology Team Research Focus-Group

Matthew Dellinger, Timothy Ehlinger, Rebecca Klaper and Joel Weinberger. 2010 Chemicals of Emerging Concern and the Great Lakes: Bibliographic Review and Discussion. Presentation to Health Professionals Task-Force: April 26, 2010

Matthew Dellinger. 2009. Using Translational Research to Create Culturally Relevant Fish Consumption Advisories. Invited Presentation at National Institute for Minamata Disease: Minamata, Japan May 15, 2009

John Dellinger, Rick Haverkate, Andrew Hickey, Matthew Dellinger, David Pritchard, Alison Farmer, Sarah Vignavong and David Petering. 2008. ‘Eat More Fish but Choose Wisely’ Risk Reduction Strategy for Michigan Anishnaabe Tribes: Preliminary Evaluation of DVD. Poster presented at Native American Health Research Conference: Portland, Oregon August, 2008

Matthew Dellinger, John Dellinger, Rick Haverkate, Mike Ripley, Nicia Lemoine. 2006.From the Lab to the Community: Helping Tribal Members Choose the Safest Fish to Eat.Abstract and poster for Annual Indian Health Service Research Conference: Albuquerque, NM.

Michael Carvan, Matthew Rise, Matthew Dellinger, John Dellinger, Daniel Weber. 2006.The Effects of Developmental Methylmercury Exposure in Zebrafish: The Potential Influences of Maternal Diet on Early Lifestage Toxicity. Abstract for 2006 Mercury Conference: Madison, WI.

Daniel Weber, Michael Carvan, Matthew Dellinger, Leigh Smith, Frederick Williams. 2006. Behavior as a Tool to Asses the Effects of Developmental Methylmercury in Zebrafish. Invited Poster at EPA Science Forum, May, 2006, Washington, DC (2nd Place Award)

Publications and Reports:

Dellinger M, Ehlinger T, Carvan M. 2012. Zebrafish Sediment Contact Assay as a Novel Tool for Integrating Human Health and Ecological Risk Assessment. In preparation for submission to Journal of Human and Ecological Risk Assessment.

Dellinger, M., L. Tofan, and Timothy Ehlinger. 2012. Predictive Analytics and Pattern Visualization for Human Health Risk Assessment. Journal of Environmental Protection and Ecology. Accepted for Publication.

Dellinger JA, Moths MD, Dellinger M, Ripley MP. 2012. Contaminant Trends in Freshwater Fish from the Great Lakes: A 20 Year Analysis. Human and Ecological Risk Assessment. Accepted for Publication.

Dellinger M, Tofan L, Ehlinger T. 2012. Predictive Analytics and Pattern Visualization for Human Health Risk Assessment. In: BENA Istanbul 2012. Istanbul Technical University, Turkey: Balkan Environmental Association, 709-723

Dellinger JA, Dellinger MJ, Yauck J. 2012. Chapter 14 Mercury Exposure in Vulnerable Populations: Guidelines for Fish Consumption. In: Mercury in the Environment: Pattern & Process, (Bank MS, ed). Berkeley, CA:University of California Press, 289-300.

Dellinger M, Ehlinger T, Carvan M. 2011. Human Health Effects Review on Chemicals of Emerging Concern, Appendix J of IJC Chemicals of Emerging Concern Workgroup Report. Ottowa, ON, CA:International Joint Comission.Contact: Joel Weiner, (613) 995-0930

Matthew Dellinger, Michael Carvan, and Timothy Ehlinger. 2011. Human Health Effects Review on Chemicals of Emerging Concern. Appendix J of IJC Chemicals of Emerging Concern Workgroup Report. Available at: http://meeting.ijc.org/workgroups/cec Contact: Joel Weiner, (613) 995-0930

Research Funding:

2013 (est)	GLNARCH VIII center grant (In preparation for May 2013 renewal) Recipient Organization: Great Lakes Inter-Tribal Council (Brian Jackson, GLNARCH Coordinator). Specified Personnel: Program Director
2012- Present	Great Lakes Inter-Tribal Council Post-Doctoral Fellow NIH/IHS/GLNARCH, Grant #: U26IHS300408A Recipient Organization: Great Lakes Inter-Tribal Council (Brian Jackson, GLNARCH Coordinator). Intern, converted to Post-Doc
2010-Present	NARCH VI Pilot Project: Prevalence of Congenital Heart Disease in Native

Americans in Wisconsin (P.I. Dr. Andrew Pelech, CHW).
NIH/IHS/GLNARCH, Grant #: U26IHS300408A
Recipient Organization: Great Lakes Inter-Tribal Council (Brian Jackson, GLNARCH Coordinator)
Specified personnel: student liaison and research coordinator

2010-2011 NIEHS Center Grant for Pilot Project: Zebrafish (*Danio rerio)* Sediment Contact Assay as a Novel Tool for Integrating Human Health and Ecological Risk Assessment (PIs: Dr. Timothy Ehlinger and Dr. Michael Carvan)
NIEHS Center Grant #: 144-PRJ44GQ4.
Specified Personnel: Graduate Research Assistant, Author of grant
Award amount: $69,869

2004-2007 Environmental Justice Grant: Communicating Fish Consumption Risks to Minorities (Hmong & Anishinaabe). (PI: Dr. David Petering)
DHHS/PHS/NIH/NIEHS, 1R25 ES011093-01
Graduate Research Assistant

2005-2007 Neurobehavioral Development in Mercury-Exposed Zebrafish. (PI: Dr. Daniel Weber) DHHS/PHS/NIH/NIEHS, PA-03-053
Graduate Research Assistant

Documentaries and Outreach:

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2005, winner top student prize Detroit Documentary Film Festival

2005, featured documentary at Wisconsin Film Festival
2005, featured on WUWM radio story, “Veterans Day”

Eat Fish but Choose Wisely. 2007. Brochure published by Agency for Toxic Substances and Disease Registry (ASTDR), 4770 Buford Hwy NE, Atlanta, GA 30341

Nindamwaa Giigoon: an Anishnaabe Guide to Fish Consumption. 2007. Educational Video Produced in conjunction with ASTDR, Intertribal-Council of Michigan, and Reel Life Films.

Cinematographer, producer, and editor: “NARCH” (working title 2009 - present)
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